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PESTICIDES IMPACT ASSESSMENT ON SURFACE WATERS BODIES OF ALMONDA SUBBASIN: AN ECOTOXICOLOGICAL APPROACH

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Dissertação para a obtenção do Grau de Mestre em

Engenharia do Ambiente

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Doutora Matilde Maria Moreira dos Santos, Investigadora Auxiliar do Instituto do Mar;
Mestre Emília Cardoso Moura da Silva, na qualidade de especialista.

Lisboa, 2008

Acknowledgments

A dissertation, concerning its academic finality, is an individual work. However, there are always people that care to help.

Therefore, I would like to express thanks to:

Professor Maria José Cerejeira, mentor of this dissertation, for her dynamism, encouragement, improvement suggestions, demands of scientific rigour, dedication presented while instructing me and for her friendship.

Engineer Emília Silva, for the unconditional support, constant availability, her valuable input and friendship.

Dr. Sara, for her vital contribution throughout all this process and for being always cooperative.

Also, a special thank to Engineer Mário Antunes and Engineer Suzete for their commitment and interest in my work and for the granted information.

Dr. Matilde Santos, from IMAR, for the opportunity of working with such a challenging research area, willingness, hospitality and the knowledge shared.

António Oliveira, who showed great altruism in the final stages of this work, and whose role made possible the conclusion of my work, namely by revising the English writing.

Ana and Dulce, for all this years of friendship and companionship.

My parents and specially my sister Inês, for their incentive and unconditional support throughout all the stages of this work, patience and great friendship. These words are not enough to express the gratitude and I will always be in debt to them.

Sandra and Luís, who were very supportive and understanding since the first day.

Fábio, you always stood by me when I needed the most, and that kept me positive that this work was possible.

Abstract

The main goal of this work is to assess the pesticides impact on surface waters bodies of Almonda Subbasin in the agricultural area of Golegã. In this area maize and potato represents the most important agricultural irrigated ecosystems.

In order to assess the waters bodies exposure to pesticides, surface water was collected in Almonda SubBasin from 5th June to 13th August of 2008. Insecticides and herbicides were detected from the monitored pesticides, particularly 3,4-DCA, alachlor, metolachlor, atrazine, terbuthylazine, chlorpyrifos, E and Z - chlorfenvinphos, ethofumesate and propanil, reaching the maximum values, respectively of, 20.2, 10.75, 1.83, 1.36, 3.41, 0.30, 0.05, 0.26, 0.16 and 7.41 µg /L.

Ecotoxicological tests were performed with the bacteria *Vibrio fischerii,* the algae *Pseudokirchneriella subcapitata,* the crustacean *Daphnia magna* and the freshwater midges *Chironomus riparius.* Results revealed toxicity only for the algae *P. subcapitata* and the crustacean *D. magna,* specifically, growth inhibition of the algae was observed in 64% of the samples and effects (immobitly/mortality) on the crustacean by for 43% of the samples.

These results confirm surface waters exposure levels to pesticides that must be reduced in the agricultural area at study.

Keywords: Pesticides; Surface water; Ecotoxicology.

Resumo

Este trabalho teve como principal estudo avaliar o impacte dos pesticidas em águas superficiais da subbacia do Almonda na qual está inserida a região agrícola da Golegã. Nesta área agrícola a batata e o milho representam os principais ecossistemas agrícolas irrigados.

Para avaliar a exposição das massas de águas a pesticidas, amostras de águas superficiais foram recolhidas na subbacia do Almonda durante o período de 5 de Junho a 13 de Agosto de 2008. Foram identificados insecticidas e herbicidas, em particular o 3,4-DCA, alacloro, metolacloro, atrazina, terbutilazina, clorpirifos, E e Z-clorfenvinfos, etofumesato e propanil, com valores máximos de concentração detectados, respectivamente de 20,2; 10,75; 1,83; 1,36; 3,41; 0,30; 0,05 0,26; 0,16 e 7,41 µg/L.

Testes ecotoxicológicos foram desenvolvidos com a bactéria *Vibrio fischeri*, a alga *Pseudokirchneriella subcapitata*, o crustáceo *Daphnia magna* e as larvas do organismo *Chironomus riparius*. Os resultados revelaram apenas toxicidade para a alga *P. subcapitata* e o crustáceo *D. magna*, sendo que 64% das amostras provocaram uma inibição no crescimento da *alga* e 43 % das amostras causaram efeitos (imobilidade/mortalidade) para o crustáceo.

Os resultados obtidos confirmam níveis de exposição das águas superficiais a pesticidas, que justificam a necessidade da sua redução na área agrícola em estudo.

Palavras-chave: Pesticidas; Águas superficiais; Ecotoxicologia.

Resumo alargado

Entre as principais preocupações ambientais a nível Mundial, a problemática em torno dos pesticidas e a necessidade de integração das questões ambientais nas principais actividades socioeconómicas, garantindo um uso sustentável dos recursos ambientais, ganharam um lugar de destaque nos principais fóruns de discussão.

Devido à sua natureza os pesticidas quando utilizados incorrectamente representam um factor de risco, quer para a saúde Humana quer para o Ambiente. Contudo, não se pode desprezar a sua utilidade nos planos económicos, em particular no sector agrícola.

Importa, assim, discutir novas medidas e/ou soluções que permitam responder positivamente a estes novos desafios, quer nas práticas agrícolas quer na comercialização dos alimentos, uma vez que a actual exigência de qualidade dos produtos agrícolas pelos consumidores não engloba apenas a garantia de produtos saudáveis e seguros, mas também que todo o processo de produção não seja sinónimo de degradação ambiental.

Representando o concelho da Golegã uma área em franca ascensão no sector agrícola, e estando localizada na região do Vale do Tejo que integra actualmente cerca de 28,3 milhares de explorações agrícolas que ocupam cerca de 7% da SAU (Superfície Agrícola Utilizada), procedeu-se à avaliação do impacte ambiental do uso de pesticidas naquela região, particularmente no compartimento água através de um estudo integrado que inclui metodologias baseadas em estudos de campo, modelação e laboratoriais no âmbito da ecotoxicologia. Pretende-se assim, que este trabalho represente mais uma ferramenta no apoio à tomada de decisão por parte dos técnicos e agricultores, e contribua para uma gestão agro-ambiental apropriada, ou seja, uma gestão do risco através da sua avaliação e caracterização, permitindo assim a discussão de medidas e gestão dos principais factores de risco.

Numa abordagem preditiva recorreu-se ao cálculo de um modelo de análise multicompartimental, designadamente o Modelo de Fugacidade de Mackay, bem como a indicadores ambientais que permitiram avaliar o risco potencial dos pesticidas para os sistemas terrestres epígeos e hipógeos e água superficial, suportada numa caracterização físico-química e de partição, ecotoxicológica e toxicológica daqueles compostos, possibilitando uma selecção *a priori* daqueles que apresentam um maior potencial de contaminação das massas de água de superfície.

Os pesticidas seleccionados neste estudo correspondem aos homologados para as principais culturas da região, designadamente a batata, o milho, hortícolas e pomoídeas.

Para a monitorização de resíduos presentes nas amostras de águas superficiais foram realizadas análises químicas qualitativas e quantitativas. Para um total de 14

amostras de águas superficiais foram identificados por microextracção em fase sólida (SPME) e cromatografia gasosa acoplada a espectrometria de massa (GC-MS) vários pesticidas e/ou metabolitos designadamente os herbicidas alacloro, atrazina, clorpirifos, etofumesato, metolacloro, propanil, terbutilazina e os metabolitos 3,4-DCA, Z-clorfenvinfos. E-clorfenvinfos.

Também foram recolhidas amostras de sedimento uma vez que estes servem simultaneamente como reservatório (sink) e fonte (source) de materiais orgânicos e inorgânicos (Griffiths, 1982). A contaminação dos sedimentos adquiriu uma importância crescente, não só pela possibilidade de transporte dos contaminantes para outros locais, mas ainda pela tendência da sua concentração naquele compartimento, onde poderão acumular-se e vir a constituir uma fonte de libertação de contaminantes a longo prazo. Este facto é sobretudo relevante no caso de compostos mais persistentes (Cerejeira, 1993).

A realização de estudos ecotoxicológicos permitiu avaliar a toxicidade aguda e crónica nas amostras de água superficial e sedimento nos organismos aquáticos *Daphnia magna*, *Pseudokirchneriella subcapitata*, *Chironomus riparius e Vibrio fisherii* uma vez que representam um papel importante na manutenção e viabilidade dos ecossistemas aquáticos.

Apenas foram observados efeitos negativos sobre dois dos organismos aquáticos em estudo, designadamente nas espécies *Pseudokirchneriella subcapitata e Daphnia magna*, sendo que para um total de 14 amostras, 64% revelaram ser tóxicas para a alga *P. subcapitata,* isto é, apresentaram uma % de inibição do crescimento superior a 50%; por outro lado apenas 43% das amostras revelaram ser tóxicas para o crustáceo *D. magna*, isto é, causaram efeitos na imobilidade e/ou mortalidade deste organismo aquático.

A avaliação da toxicidade do sedimento proveniente da zona de Alverca do Campo foi realizada com base nos efeitos no crescimento e sobrevivência – *endpoints* – no organismo *Chironomus riparius,* cujos resultados obtidos traduzem a ausência de toxicidade para este organismo quando exposto ao sedimento em estudo.

Integrando os valores de toxicidade e exposição, concluiu-se que, para os níveis de efeito e concentração de pesticidas obtidos não seria possível estabelecer uma relação entre os níveis considerados. Para além de que, confrontando os níveis de resíduos doseados de pesticidas nas amostras em estudo com valores de toxicidade referidos em bibliografia específica, concluiu-se que a diferença entre entres é suficientemente grande para não ser possível estabelecer relações de dose-efeito.

Os valores de toxicidade obtidos poderão assim estar relacionados com a presença conjunta destes pesticidas e ou outros pesticidas, ou eventualmente de outros compostos presentes nas massas de água em estudo, pelo que constitui um tema a considerar em futuros trabalhos, uma vez que resultados em testes provam que a co-ocorrência de

compostos poderá influenciar as propriedades individuais de cada um, ou seja, a toxicidade da mistura poderá ser superior à dos compostos individuais mesmo quando presentes individualmente em concentrações superiores do que as em mistura (Stackelberg *et al*., 2001).

Os resultados obtidos neste trabalho confirmam de facto níveis de exposição das águas superficiais a pesticidas que justificam a necessidade da sua redução. Evidencia-se ainda a necessidade de uma utilização sustentável dos pesticidas, para a qual é exigível um reforço da investigação na área dos pesticidas no ambiente, a monitorização contínua e a integração multidisciplinar de metodologias e medidas com vista à gestão do risco, especialmente em zonas de elevada susceptibilidade à contaminação por pesticidas e com forte pressão agrícola.

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Acronyms

ADI Acceptable Daily Intake

ASTM American Society for Testing and Materials

B Bioaccumulation

BCF Bioconcentration factor

BMPs:Best Management Practices

CAP Common Agricultural Policy

C_o phase of octanol

C_w aqueous phase

DC Diet Concentration

D_F Drift Fraction

DGADR "Direcção-Geral de Agricultura e Desenvolvimento Rural"

DL Decree-Law

DQA "Directiva Quadro da Água"

DT_{50} soil Dissipation time

EAP Environment Action Programme

EC_{50} Median effective concentration

EC European Community

EEC European Economic Community

EPA Environmental Protection Agency

EPPO European and Mediterranean Plant Protection Organization

EU European Union

FAO Food and Agriculture Organization

GC-EI-MS Gas Chromatography – Electronic Impact – Mass spectrometry

GC-MS Gas chromatography-mass spectrometry

GR Growth rate

H Henry´s law constant

HQ Hazard quotient

IMAR "Instituto do Mar"

INAG "Instituto da Água"

INE "Instituto Nacional de Estatística"

K_{aw} Partition coefficient air/water

K_oc Organic Carbon Sorption Coefficient

K_{ow} Partition coefficient octanol/water

LD_{50} Median lethal dose

LOEC Lowest Observed Effect Concentration

LRA "Laboratório de Referência do Ambiente"

MRLs Maximum Residue Limits

MRA Maximum Application Rate

MT Megatons

NOEC No observed effect concentration

NOEL No Observed Effect level

OD Optical Density

OECD Organisation for Economic Co-operation and Development

PEC Predicted Environmental Concentration

PED Predicted Environmental Distribution

PENDR "Plano Estratégico Nacional de Desenvolvimento Rural"

RNPB "Reserva Natural do Paul do Boquilobo"

SPME Solid-phase microextraction

TDI Total Daily Intake

UAS Utilized Agricultural Area

UNESCO United Nations Educational, Scientific, and Cultural Organization

USEPA United States Environmental Protection Agency

VOC´S Volatile Organic Compounds

ZVT "Zona Vulnerável do Tejo"

% Percentage

> greater than

< less than

= equal to

1. Introduction

Agriculture is an economic sector that depends upon a role of natural sources as production factors: the soil, water, air and the genetic patrimony.

Today we face challenges concerning water resources, both in our own country and worldwide.

By the turn of the century, the impact on surface water is evident; and the dumping of sewage effluent by-products of manufacturing and agriculture had become associated with the terms contamination and pollution. Consequently, using pesticides, effectively, while maintaining water quality, presents an important challenge.

Surface waters are a precious resource for life preservation and a vital component for all the global ecosystems.

Some 70% of the Earth's surface is covered by seas and oceans, and these produce almost three quarters of the oxygen we breathe and only 1% of this water can be used directly; however 20% of the surface waters in European Union are at risk because of pollution, and many forms of human activity put water resources under considerable pressure.

Chemical pollution of surface water can disturb aquatic ecosystems, causing loss of habitats and biodiversity. Pollutants, specifically pesticides may accumulate in the food chain, and harm predators consuming contaminated fish. Humans are exposed to pollutants through the aquatic environment by fish or seafood consumption, drinking water and possibly recreational activities. Pollutants may be found in the environment many years after being banned; some may be transported long distances and can be found in remote areas (European Commission, 2002).

The term pesticide refers to a large number of diverse chemicals employed to control one or more species deemed to be undesirable from the human viewpoint.

Since the early 1960's, when the negative environmental impacts of pesticide use became a topic of societal debate, an increasingly refined and detailed regime of measures was implemented in order to reduce environmental impacts of pesticide use (Hond *et al*., 2003). They are environmental concerns for two main reasons: although considerable progress has been made with respect to their selective toxicity as mentioned above, many still possess significant toxicity for humans, and many persistent poisons, so that biological features allows bioaccumulation and biomagnifications up the food chain; and there is the possibility that they may enter human food supplies, as well as constitute an ecological hazard (Philp, 1995).

Sustainable agriculture includes sustainable pest management. But how sustainable are current pest management practices that rely heavily on the use of pesticide products? (Hond *et al.,* 2003)

Attitude to pest management became polarized over the final three decades of the 20th century. Prior to this, in the 1950´s and 1960´s, pesticides were seen by many as a panacea for pest problems. However, a reappraisal of the role of pesticides and a "rediscovery" of the importance of biological control mechanisms started until nowadays (Wilson, 2003).

It is probable, if not certain, that pesticides will continue to play a vital part in the safe and economic production of food in the foreseeable future. Notably, outside the developed world, pest control strategies, including the use of chemicals, are essential for adequate food production and for current human health strategies (Wilson, 2003).

Today´s, pesticide products are high-tech products: considering the systemic character of many fungicides, the low-dose rates of several herbicides and the specificity of insecticides. They exhibit higher selectivity and reduced persistence as a consequence of the introduction of new active ingredients and formulations (Hond *et al.*, 2003). However, as mentioned above, they continue to appear in surface waters.

The European Union (EU) policies and the Common Agricultural Policy (CAP) are aimed at the eradication of risks for the environment degradation, fomenting a positive idea about agriculture's contribution for the natural spaces and environment preservation through specifically rural development actions.

The present panoramic, regarding to the hydrologic resources' protection specifically to surface waters, illustrates the positive effects of the initiatives endorsed until now in terms of legislation.

However, the European Union acknowledges that to face current environmental challenges a strategic approach must replace a strictly legislative one, by resorting to diverse instruments and measures to influence the ones who make decisions: entrepreneurs, politicians, consumers and citizens. As a result, overtime, agricultural priorities have changed, such as concerns over food safety, health and environmental, which have become more prominent.

In 2006, a Eurobarometer survey studied consumers' attitudes towards agriculture and the CAP. The results express the consumer´s concerns to healthy and safe products, a fair standard of living for farmers and reasonable prices for consumers and more important respect for the environment (Figure 1.1).

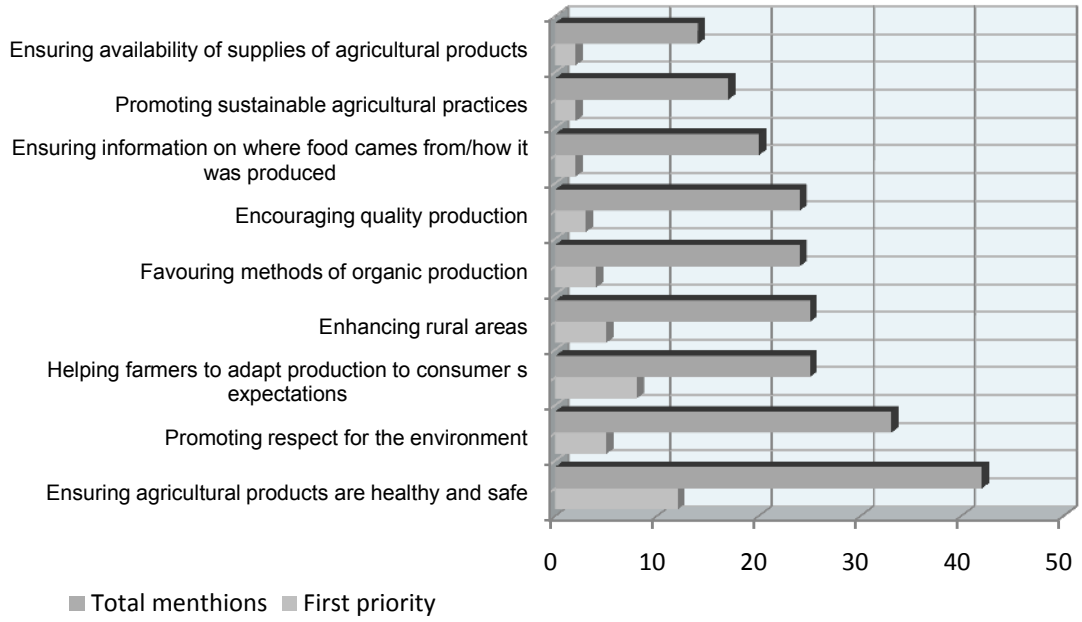

Figure 1.1 - *Priorities for European agricultural policy, EU-25, 2006 (adapted from Eurostat: 2008)*

In this perspective and considering the environment parameters that the "production quality" includes, it is important to considerer the impact agriculture practices evaluation, namely the pesticides impact usage in the hydrological and sediment resources.

Managing the risk or effects of pesticides requires far more information than we can afford to directly measure for all the places and all the times, and all the pesticides of interest. Strategies and/or tools are therefore required to focus monitoring and risk assessment programs in a cost-effective manner, and to predict pesticides concentrations and effects. To access the risk of pesticides in aquatic ecosystems information is required on the environmental fate of pesticides, their concentrations in the environment (exposures) and toxicity to aquatic organisms. The overall ecological risk can then by determined based on the general principle that risk is a function of toxicity and exposure (Figure 1.2) (DPI, 2007).

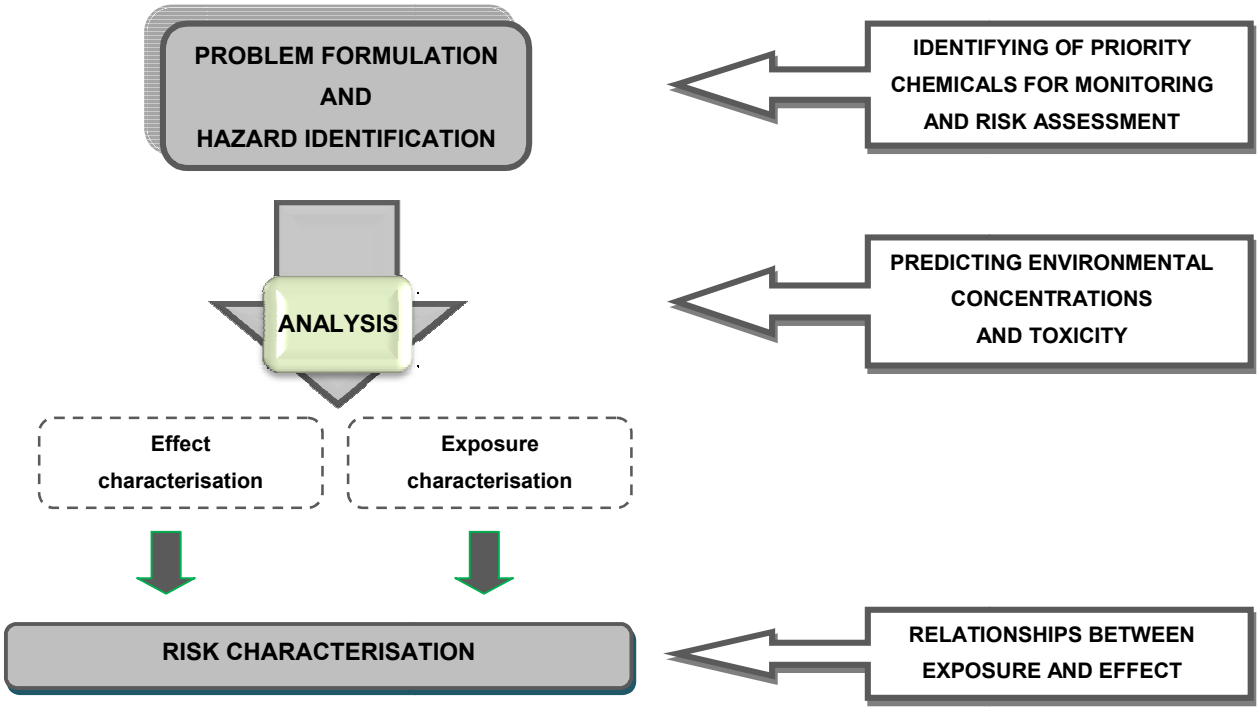

Figure 1.2 - *Risk assessement steps (DPI, 2007).*

The agricultural area at study in Almonda subbasin, in previous studies, showed to be a vulnerable area to pesticides contamination. Today, problems linked to pesticides surface waters contamination are still affecting this area. Being under a high-pressure agricultural area, it is important to continue assessing the pesticides impact in terms of exposure and effects studies based upon the risk management.

For this purpose, in an integrated approach, this work can be distinguished in three main stages:in the 1st stage the selection of the most important crops in the study area, as well as, the list of the pesticides registered for these same crops and the physical-chemical and partition properties characterisation. Secondly, the 2nd stage includes the environmental exposure *a priori* and potential hazard assessment for the different environmental compartments (short and long term), based on Mackay´s fugacity model and environmental indexes (EPRIP). The final stage includes the surface water exposure levels to pesticides and toxic effects studies on aquatic organisms in surface waters and the potential hazard assessment of pesticides in surface waters of the study area.

Considering all this approaches, it is possible to contribute to a better management and use of pesticides and provide useful tools to technicians and farmers in the region.

In chapter 2 the behaviour and fate of pesticides in surface waters is discussed. Chapter 3 refers to the role of ecotoxicology in the hazard assessment. Chapter 4 (Environmental impact assessment of pesticides in the Almonda sub-basin) is introduced by a general description of the area in study in order to understand the impact of agriculture in this area. Focusing on the pesticides registered for the crops at study, specifically the potato, maize, horticultures and fruit trees, predictive approaches and methodologies of sampling and analytical methodology to surface water exposure assessment to pesticides, and bioassays to assess the toxicity on surface waters and sediments for the nontarget organisms is described. This chapter also includes the results obtained in laboratory and its discussion, in an attempt to confront these results with what was expected theoretically.

In the chapter 5, some measures for the pesticides impact mitigation on surface waters are suggested. Finally, in chapter 6 there is a brief sum up of the principal ideas to take into to account presently and a reference for future studies on this thematic, the pesticides impact on surface waters bodies.

2. Pesticides in surface waters

2.1. Behaviour and fate of pesticides in surface waters

Water is one of the primary mechanisms by which pesticides are transported from applications areas to other parts of the environment, resulting in the potential for movement into and through all components of the hydrologic cycle. Surface waters are particularly vulnerable to contamination by pesticides, because most agricultural drain into surface water systems (*Larson et al., 1997)*.

Surface water is linked to both groundwater and atmospheric water through the hydrologic cycle (Figure 2.1). Surface water moves into groundwater by infiltrating the soil and percolating downward; it also enters the atmosphere through evaporation and transpiration. Likewise, water from the atmosphere and groundwater can recharge surface waters first by atmospheric waterfalls as precipitation: rain, sleet, hail, and snow and secondly by groundwater that moves to the earth's surface contributes to the base flow of streams, lakes, wetlands, and other waterways (Whitford *et a*l., 2001)

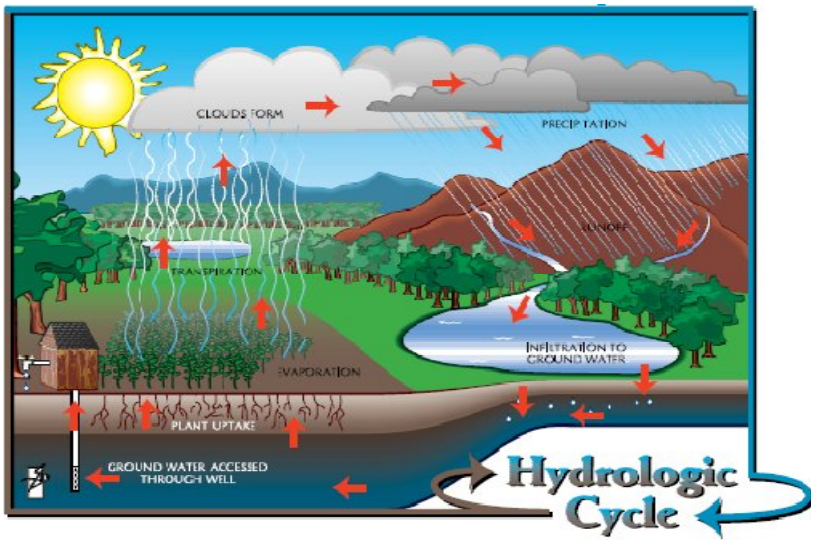

Figure 2.1 - *The hydrologic cycle (Whitford et al., 2001).*

Pesticides can enter water bodies via diffuse or via point sources. Point and diffuse sources contribution to pesticides pollution in river systems was demonstrated to be very important in several catchments in Europe. Diffuse and point sources are not unequivocally defined in the literature, and often a clear distinction between the two is not possible. According to Reichenberger *et al*. (2007) diffuse-source pesticide inputs into water bodies is defined as the inputs resulting from agricultural application on the field. In contrast, point source inputs derive from a localized situation and enter a water body at a specific or

restricted number of locations. For example, high pesticides concentrations during dry periods indicate point sources. The spill during filling of the spraying equipment, cleaning of the equipment and processing of spray waste on paved surfaces are examples for bad management practices. As diffuse input pathways, runoff, drainflow, drift, atmospheric deposition and groundwater flow can be distinguished (Holvoet *et al.,* 2007*).*

A complex environmental behaviour is observed after pesticides appliance, such as physical, chemical and biological processes. This dynamic influences the pesticides distribution in different compartments (soil, water, sediment, biota and air).

The use of pesticides in agriculture may lead to contamination of surface water by drift, runoff, drainage and leaching (Figure 2.2).

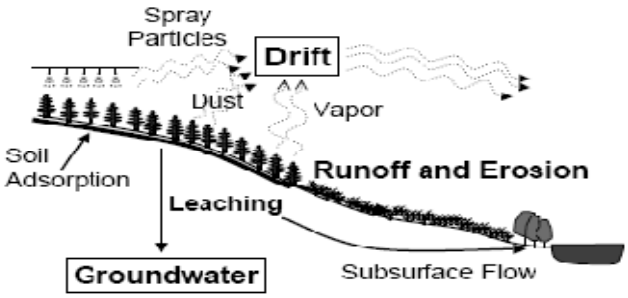

Figure 2.2 - *Pesticide movement (adapted from Bosworth, 2008).*

Surface waters contamination depends on factors such as: climate (temperature, light, precipitation, wind's velocity); pesticides application (intrinsic products properties, application method) and other agricultural practices (soil mobilization, irrigation); soil characteristics (for example: texture, pH, organic matter content) and the hydrologic vulnerability of the area (Cerejeira, *et al*., 2002).

Understand the fate of pesticides requires an understanding of certain processes: transformation; transfer; and transport.

- Transformation refers to biological and chemical processes that change the structure of a pesticide or completely degrade it.
- Transfer refers to the way in which a pesticide is distributed between solids and liquids (e.g., between soil and soil water), or between solids and gases (as between soil and the air it contains).
- Transport is the movement from one environmental compartment to another, such as the leaching of pesticides through soil to groundwater; volatilization into the air; or runoff to surface water (Withford *et al*., 2001).

The study of pesticides mobility in the environment, i.e., the potential assessment, destiny and environmental behaviour of pesticides, as well the toxic effects (before introducing them in the environment) are an extremely important procedure.

Attempting to identify for each pesticide the most at risk environmental compartments, mathematical models have been developed. The Mackay´s Fugacity Model is one of the most important evaluative models and is simple and easy to handle and requires few input data (Vighi & Di Guardo, 1995). More details are expressed in chapter 4.1.3.2 of the present work.

2.2 National studies on surface water exposure assessment to pesticides

The first pesticides residues analysis started in 1983 on Tagus River allied to the Portuguese Program of Water Quality establishment, in order to acquire surface waters quality situation and evolution tendencies and understanding the nature of human and natural factors potentially capable of affecting the aquatic system.

Between 1983 and 1993, the presence of residues above 1 µg/L was registered, specifically organochlorines insecticides in 14% of the water samples, with special attention to lindane and alpha-endossulfon; between 1990 and 1993 herbicides and organophosphates insecticides were detected in 24% of the water samples, with highlight for the herbicides atrazine, simazine and molinate and the insecticide chlorfenvinphos (Amaro, 2003).

Furthermore, the “Secção de Protecção Integrada” from the “Departamento de Protecção das Plantas e de Fitoecologia” from “Instituto Superior de Agronomia” had been involved in a number of large-scale studies of groundwater exposure levels to pesticides, as well as of surface waters and the pesticides effects on the aquatic system.

From 1996-1998 under the project PAMAF 4024 studies began to cover almost all areas in the entire land of DRARO (“Direcção regional de Agricultura da Região Oeste”), also extending the range of products evaluated (Batista *et al*., 1998, 2001; Cerejeira *et al*., 1999 a, b, 2003; Silva-Fernandes *et al*, 1999).

Among 2004 to 2006 occurred the project Agro 530 that aimed to complement a decision support system in the vulnerable zone of the Tejo (Mendes *et al*., 2006; Duarte, 2004; Rei, 2005; Barros, 2005; Basto, 2006). Within these studies were published several works both at national and international level (Batista *et al*., 1999 a, 2000 b, 2001, 2002; Cerejeira *et al*., 1998 a, 2003; Silva-Fernandes *et al*., 1999).

All of these studies (including Barros (2005) and Basto (2006)) targeted triazine concentrations inferior to 0.1 µg/L, and some included other high-use pesticides, like the insecticides lindane, alpha-endosulfan, as the herbicides atrazine, simazine, molinate and chlorfenvinphos (Z+E), with maximum dosed concentrations of 1.65 ug/L, 0.032 ug/L, 0.63 ug/L, 0.294 ug/L, 1.5 ug/L e 0.298 ug/L, respectively.

2.3 Legislative aspects – pesticides and water

2.3.1 Placing of plant protection products on the market

In the past plant protection products were introduced in the market without a previous analysis and/or registration process, until 1967, the year of the publication of the D.L. 47802, of July 19th. This D.L. established that plant protection products would only be marketed after a rigorous registration process, including studies on pesticides behaviour in the soil and water compartments.

However the real turning point has to be associated with the introduction of risk analysis divided in three components: assessment, management and communication of risk as a consequence of the Directive 91/414/EEC, of 15 July 1981, concerning the placing of plant protection products on the market which has harmonized the conditions and procedures for authorising this products so as to protect human health and the environment.

This Directive that became effective in July 26th of 1993 and it was transposed to the national law through the D.L. No 284/94 of November 11 and the ordinance No 563/95 of June 12, presents new demands, procedures and evaluation and decision criteria for the new active substances and plant protection products as well for the substances already on the market.

2.3.2. Towards a thematic strategy on the sustainable use of pesticides

"Optimizing pesticide use" is a very broad phrase that can be interpreted in a number of ways. According to the European Community Commission, the sustainable use of pesticides is defined as: "the use of pesticides without irreversible effects in the natural systems and without acute and chronic effects for men, animals and environment. A sustainable use leads to the greatest use reduction of pesticides, the soil restriction or the substitution of the highest dangers and the adoption of the Precaution Principle in the pesticides homologations decisions" (European Commission, 2001).

It is probable, if not certain, that pesticides will continue to play a vital part in the safe and economic production of food in the foreseeable future. Notably, outside the developed world, pest control strategies including the use of chemicals are essential for adequate food production and for current human health strategies (Wilson, 2003).

The EU acknowledges that to face today´s environmental challenges, a strategic approach must replace a strictly legislative one by resorting to diverse instruments and measures. In adopting the 6th Environment Action Programme (6th EAP), the European Parliament and the Council recognised that the impact of pesticides on human health and the environment, in particular from plant protection products must be further reduced. They underlined the need to achieve a more sustainable use of pesticides as well as a significant overall reduction in risks and of the use of pesticides consistent with the necessary crop protection and with the principle of sustainable development. Also, aiming for the improvement or the maintenance of the current state of the biological diversity conservation and stop its reduction due to agricultural activity factors, the EU has adopted in March 2001, the plan for Action in terms of Biodiversity for the agricultural sector.

The interaction between the needs of agriculture, environmental protection and concerns for human health is complex, and is depicted in figure 2.3.

Figure 2.3 - *The interaction of the principal factors in the pest-control practices (Wilson, 2003).*

Taken together the combined improvements in chemistry, application technologies and the chemical, physical and biological aspects integration with each other, one can genuinely envisage an optimization in pesticide use without compromising the quality and efficiency of farming or consumer and environmental protection.

Sustainable agriculture integrates three main goals - environmental health, economic profitability and social and economic equity. Sustainability is based on the principle that we must meet the present needs without compromising the capacity of future generations to meet their own needs. Consequently, the overall environmental aim of sustainable

agriculture is to optimize the use of natural resources while at the same time maximizing the efficiency of input use and preserving environmental integrity (Feenstra, 1997).

The Integrate Protection Principle and also integrated production is stringently related with the sustainable agricultures concept. The integrated production was developed as a farming system capable of meeting the requirements of the long-term sustainability (Anipla, 2008). The main goal of the Integrated Protection is to fight the crops enemies in a economic way, efficiently and with the lowest inconveniences to the Men and environment, based upon a rational use, equilibrated and integrated of the all available fight resources (genetically, cultural, biologic, biotechnique and chemical), with a level that the crops enemies do not cause damages (Amaro, 2003).

2.3.3. Water protection and management

As global projections for water demand and availability point towards increasing scarcity, water resource managers and policy makers are looking for more innovative strategies to increase water use and allocate efficiency, as well as to manage, demand through provision of efficiency-enhancing incentives (Msangi *et al.*, 2005).

At the present the principal protection mechanism for water in the European Union is the Water Framework Directive 2000/60/EC of October 23t^{h} – the most important Directive in the environmental sphere of action.

This Framework Directive provides, among other things, the European waters identification and their characteristics on the basis of individual river basin districts, and the adoption of management plans and programmes of measures appropriate for each water body.

The Water Framework Directive introduced on updated, comprehensive and effective strategy for the chemical pollution of surface water. Some of the target objectives in order to protect surface waters are:

- The deterioration prevention, improvement and re-establishment of surface waters' masses conditions;
- Guarantee a high-quality (chemical and ecological) of surface waters until 2015;
- The reduction of pollution resulting from discharges and emission of priority substances
- Promoting the surface sustainable usage, attending an integrated management in a long term.

Priority substances in the field of water policy have been defined after the decision 2455/2001/EC of the European Parliament and the Council from 20th November, 2001 (amending Directive 2000/60/EC), which includes a list of 33 priority substances, specifically 13th pesticides - alachlor, atrazine, chlorfenvinphos, diuron, endosulfan, HCH, hexachlorobenzene, isoproturon, lindane, pentachlorophenol, simazine and trifluralin – being the progressive reduction of discharges, emissions and losses of this substances the main goal.

The Water Framework Directive required the establishment of environmental quality standards applicable to water. The best way to achieve a good surface water chemical status in the European Union is to harmonise the environmental quality standards that exist at national level for priority substances. Therefore, the Commission proposed establishing environmental quality standards so as to limit the quantity of certain chemical substances that pose a significant risk to the environment or to health in surface water in the European Union (EU) established by the Proposal of 17 July 2006 for a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2000/60/EC. The purpose of this proposal is to set out environmental quality standards concerning the presence in surface water of certain pollutants and substances or groups of substances identified as priority on account of the substantial risk they pose to or via the aquatic environment.

Concerning to the protection of groundwater against pollution the European Commission presented, in September 2003, a proposal for a directive of the European Parliament and Council on the protection of groundwater to prevent and control pollution of this resource. As regard to pesticides and its metabolites the proposal set the value of 0.1µg/L as a quality standard for groundwater. The named “daughter directive”, Directive 2006/118/CE of the European Parliament and the Council, of December 12, 2006, relative to the protection of groundwater against pollution and deterioration implements criteria for the assessment of good chemical status groundwater, and criteria for identifying and reversal of trend

significant and sustained upward concentrations of and for the definition of points of starting that trend, under Article 17 of the Water Framework Directive. According to Article 2, the applicable linear for a good groundwater chemical status should be based on protection of water mass with particular attention to the impact on, and its interrelation with, the surface waters and associated ecosystems and wetlands directly dependent. Quality standards defined as criteria are congruent with the Council Directive 91/414/EEC on the placing of plant protection products on the market, and Council Directive 98/83/EC on the quality of water intended for human consumption.

3. The role of ecotoxicology in the hazard assessment

The idea that human health cannot be protected unless in conjunction with wildlife protection, led to a definition of a new branch in environmental sciences: *ecotoxicology*. The term was defined by Truhaut (1995, 1997) and later by Butler (1978) as the branch of toxicology that studies the toxic effects of natural and artificial substances on living organisms (Rand, 1995).

Producing criteria for the prevention or contamination reduces, is the main goal of ecotoxicology. Due to the fast technology evolution, ecotoxicology should also be applied to predictive instruments in order to produce criteria even for hypothetical chemicals or potential contaminants (Bacci, 1994).

The hazard identification and the risk assessment, as well as its characterization are the risk assessment basis. However, the ecosystem diversity requires an ample planning which includes problem identification, its analysis and risk definition (Amaro, 2003).

Ecotoxicological effects, as the result of use of pesticides may be direct or indirect, and can been measured through laboratory acute and chronic toxicity tests or by observing organisms effects in field (Rand, 1995).

In ecotoxicology another important aspect is the analysis of concentration levels of chemical compounds in different environmental matrices, i.e., the concentration of the chemical compounds that organisms are exposed to a given environment. The evaluation of exposure can be done using mathematical models or through monitoring studies.

The exposure assessment also involves the analysis of properties of the compound to determine its fate and transport, and modeling the processes of transport, like the distribution potential of molecules in the environment (PED- Predicted Environmental Distribution) determination (Pereira *et al*., 2003). Those contribute to the assessment hazard of the potential risk of these products (Cerejeira, 1993).

Aquatic toxicology is part of the ecotoxicology science, which is multidisciplinary in scope and interdisciplinary in practice.

The aquatic environment vulnerability for pesticides depends on several factors, including (1) physical and chemical properties of the pesticide and its transformation products; (2) concentration and total loading of the pesticide entering the ecosystem; (3) inputs period and type; (4) ecosystem properties that enable it to resist changes, that could result from the pesticides presence and (5) ecosystem location, relative to point source of pesticides. Because aquatic ecosystem involve complex interactions of physical, chemical and biological factors, it is difficult to understand the system response to pesticides, unless the relationships among components of the system are well defined (Rand, 1995).

4. Environmental impact assessment of pesticides in the Almonda subbasin

4.1. Material and methods

4.1.1. Study area characterisation

4.1.1.1. Location – Almonda sub-basin

For this study surface waters quality assessment was performed on Almonda subbasin – Tagus alluvium aquifer system which occupies a total area of 1113 Km^2 - in particular at "Alverca do Campo" and in two different location of Almonda river, specifically upstream and downstream of the "Reserva Natural do Paul do Boquilobo" (RNPB), respectively "Ponte do Paul" and "Quinta da Broa" (Figure 4.1).

The Almonda river is a tributary of Tagus river in the right margin, located between Aviela and Zêzere rivers, with a total area of 228.6 Km^2. Although not being the most representative in the basin total area, not only the Almonda river continues to flow to Tagus river, also in flood periods the Tagus and Almonda rivers drain to the RNPB. This area integrates the Tagus-Sado basin Hidrogeological unit that covers a total surface of 80629 Km^2, of which 24800 Km^2 are located in Portuguese ground, where there is a 3.8 million population (Bastos, 2006).

Figure 4.1 - *Study area: "Alverca do Campo" and Almonda River upstream (AlmondaR-1) and downstream (AlmondaR-2).*

The area at study belongs to Santarém district, located in the centre of the country, more specifically in the fertile "Vale do Tejo", one of the geographic areas of the country with more agro-pecuary production relevance and one of the best flood plains of Europe.

4.1.1.2. Soils and hydrology

With a vast utilized agricultural surface (UAS), of approximately 7% out of the national whole and with a forest area of 17% out of the total of the continent, the Ribatejo lands hold, as previously stated, unique natural conditions for the development of the agricultural sector (INE, 2001).

According to the "Carta de solos de Portugal" and the FAO scheme, the soils with greater relevance in "Campo" are: Eutric Fluvisols (Je) and Calcaric Fluvisols (Jc).

Considering the hydraulic system, the modern alluviums represent the recharge area where the profound infiltration of rainwater takes place; concerning to the discharge system, these occur across the central water lines; regarding to the flow directions and the subterranean flow off, these occur towards the Tagus River and across modern alluviums stripe, ending in the Estuary (INAG, 2001).

Notice that the exploration of the alluvium aquifer system contemplates either the public supply or the industry and agriculture. Since the agriculture is the most pressured activity in the region, the exploration for this purpose is specifically vital in the area of study, with prominence to the irrigation areas where there are hundreds of wells for the extraction of subterranean water for irrigation.

The "Zona Aluvionar Norte do Tejo" was considered a vulnerable zone, according to DL 1100/2004 of September 3rd of 2004, in which the Decree-Law 235/97 of September 3rd altered by the Decree-Law nº 68/99 of March 11th, establishes the legal regime designed to protect the waters from pollution caused by nitrates of agricultural origin to the zone then designated of "Zona Vulnerável do Tejo" (Figure 4.2.) This vulnerability has been showed not only in terms of nitrates but also of pesticides, since 1993, and specifically during the AGRO 503 project in 1993.

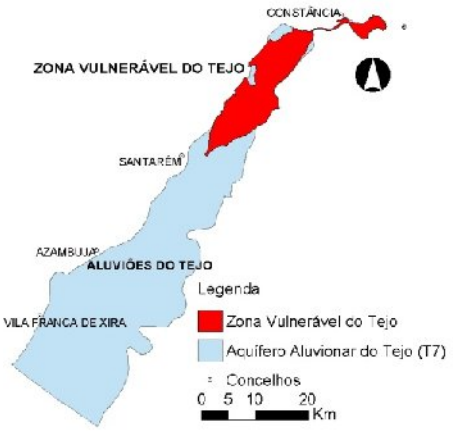

Figure 4.2 - *Localization of the ZVT in Tagus alluvium aquifer system.*

4.1.1.3. Terrestrial and aquatic ecosystems associated

The "Reserva Natural do Paul do Boquilobo" (RNBP) is one of the most important protect area in Tagus Basin, created by the Decree-Law no. 198/80 of 24th June and re-classified by the Regulamentar Decree no. 49/97 of 20th November.

Since 1981 and 1996, this whole area was classified as, respectively, Biosphere Reserve (UNESCO) and Humid Zone with International Importance (RAMSAR Convention).

With the publication in the Decree-Law no. 348-B/99 of 23rd September of the Directive 79/409/CEE, concerning to wild birds conservation, a "Zona de Protecção Especial do Paul do Boquilobo" (ZPEPB) was created, with an area of 432.78 ha. In 1991 the Natural Reserve was defined as Corine Biotope C21400012, Natural Reserve of Paul do Boquilobo.

The total area of the RNPB, specifically 554 ha, is inserted on Golegã district (which inclues Azinhaga), Torres Novas (Riachos, Alcorochel, Brogueira and Boquilobo) and Santarém district (Pombalinho and Mato de Miranda).

UNESCO, by the Program MAB (Man and Biosphere) was recognized the natural values of this area, being included in "Rede de Reservas da Biosfera". The main goal of this Project was the definition of equilibrium between technologic development and natural resources use (Pereira, 2004).

The ZPEPB represents, in its whole a vast set of significant natural values of great fauna and flora productivity. In the alluvial prairie the predominant flora is represented by ash trees and willows, supported by complex water mains, "valados" and drainage ditches and Almonda River. Prevailing as well there are the cork oak, the zambujeiro, the holm oak, the carvalho-cerquinho, as well as diverse endemic species included in the Red Book of Portugal plants and non-native species with infesting character, for example "jacinto-de-água" (*Eichornia crassipes*) and "Figueira-do-inferno" (*Datura stramonium*).

According to the "Plano de Ordenamento para a Reserva Natural do Paul do Boquilobo" (ICN, 2004) a total amount of 317 species are identified, being the majority of them are well adapted to the lack of soil ventilation, humid zones characteristic. The predominant vegetation in this Reserve is associated to humid environments, being under the influence of the hydric regime. Considering the climate conditions, flooded soils and its geographic location, they create outstanding conditions for the refuge and sustenance for many species.

Although the great biological diversity in the RNPB, the "avifaunística" component is undoubtedly the most abundant and of greater value for the preservation; reason why it was nationally recognized for the creation of a Protected area and internationally for its inclusion

as Special Protection Zone and Important Bird Area in Europe. With about 221 species, the birds represent 77% of the existent vertebrates.

Paul do Boquilobo, being a humid zone has a high importance in the hydric regulation of the region, as it works like a retention area: exceeding water accumulation during floods and aquiferous recharging during dry periods. However, the natural silting of tilled plain, as well as the practice of an intensive agricultural through the soil drainage and subterranean water bombing cause the progressive disappearing of this type of system.

The pollution levels in Paul do Boquilobo are a consequence of urban and industrial effluents from Riachos, Entroncamento and Golegã, which drain directly to the Paul's west side and the pollution of agricultural origin (nutrients and plastics). Another identified problem is the inefficient sewerage of the Almonda River Basin. Except for the agricultural activity, all the polluting causes are out of the RNPB limits (ICN, 2004).

4.1.1.4. Agricultural activities

"Ribatejo e Oeste" region, area at study, presents, according to Agricultural General Census of 1999 (INE, 2001), a UAS of 447863 ha. The irrigation surface occupies 34.5% of the UAS area (154518 ha), where the most representative of irrigated crops are maize, vegetables, fresh fruits, tomato, potato and rice.

According to INE statistic data (2001), the main water sources for irrigation included mainly wells and rivers. Today, the situation remains similar to those obtained in 1999. According to AGROTEJO (farming association of "Norte do Vale do Tejo") data, in 2006, the Golegã region had an agricultural exploration area of approximately 18938.09 ha, for a total of 573 agricultural explorations. The most representative crops in terms of % in Golegã are identified in the figure 4.3.

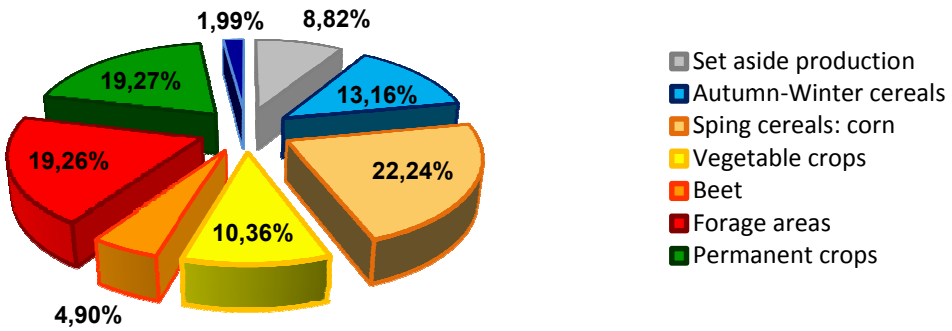

Figure 4.3 - *Main crops in 2006 in terms of total area % (adapted from AGROTEJO, 2006).*

Relatively to the presented data in the *Figure* 4.3, notice that part of the permanent crops 2093 ha are forestry; In general, and according to previous data irrigated crops are those with greatest expression in terms of production. In this work and after analysis of the dates mentioned above the following crops were considered: vegetables, apple and pear tree, maize and potato, with a particular attention to the cultures of potato and maize due its importance for this area (Figure 4.4).

Figure 4.4 - *Principal crops in the area of study: potato and maize crops respectively.*

4.1.1.5. Use of pesticides

Recently an ongoing pesticides sales increase has been observed in Portugal. This tendency has been influenced by the positive conditions for the development of diseases and weeds in vine, potato, tomato, vegetables and maize crops. The intensity of plant protection product consumption among the EU 27 Member States was highest in Portugal (predominantly in the use of fungicide products) (EUROSTAT, 2008). In 2007, approximately 14 Ton was sold, having the fungicides the highest sales % (*Figure 4.5*).

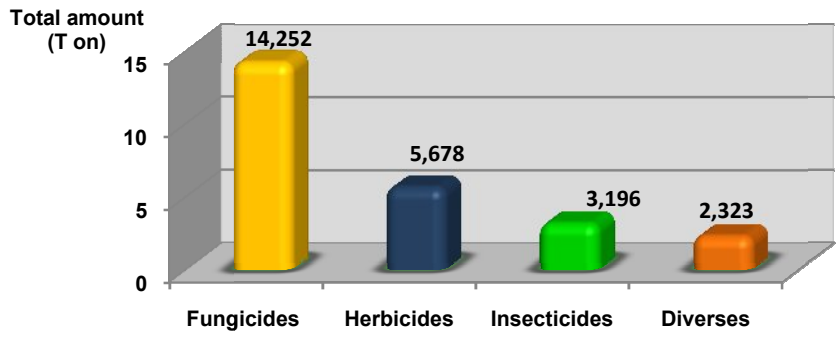

Figure 4.5 - *Quantities of sold pesticides in Portugal, year 2007, expressed in Ton (adapted from ANIPLA: Associação Nacional da Industria para a Protecção das plantas, 2008).*

From a national perspective the agricultural use of pesticides is heaviest in regions with intense agricultural activity and regions where specific crops grown.

Once the obtained data were not as exact and exhaustive as the main goal, it was only possible to define the principals pesticides used in the area in study in terms of quality.

The table 4.1 resumes the pesticides that are used more frequently in qualitative terms, for the maize and potato crops in the AGROTEJO and AGROMAIS influenced area. As mentioned above, the maize and potato crops are the most important in this region.

Table 4.1 - *Pesticides used in maize and potato crops in Golegã.*

	INSECTICIDES	FUNGICIDES	HERBICIDES
POTATO	chlorpyrifos chlorpyrifos-methyl ethoprophos lambda-cyhalothrin thiacloprid thiamethoxam	cymoxanil dimethomorph fluazinam mancozeb metalaxyl-M zoxamide	deltamethrin
MAIZE	carbofuran chlorpyrifos endossulfon imidacloprid lambda-cyhalothrin		atrazine bentazone mesotrione nicosulfuron S- metolachlor sulcotrione

4.1.2. Pesticide selection to study

Physical-chemical pesticides properties influence the exposure of surface waters in agricultural ecosystems (Bacci, 1994; Mackay *et al*., 1997). Therefore, the knowledge of properties and environment partition for each pesticide is an important component for the evaluation of pesticides behaviour and their potential distribution in the environment, for balance conditions.

With basis one the main crops for the study area considered in this work (potato, maize, vegetables, apple and pear tree), was made a review of pesticides registered for the same, totalizing 146 pesticides in 2008 (DGADR, 2008).

The physical-chemical and environment partition properties, as well as the ecotoxicological and toxicological endpoints were compiled from specific literature, in particular Tomlin (2006) and an online "Footprint" database (http://www.eu-footprint.org.), that is a comprehensive

database holding data on environmental fate and ecotoxicological properties for a large number of pesticides and their metabolites, including all those registered in Europe.

For the pesticides in study, data was collected relative to: molar mass, melting point, water solubility, vapour pressure, $\log_{Kow}$, K_oc, and DT_{50} soil at a temperature of 20º C, as well (Annex B and C).

In Annex A are presented the physical-chemical properties and in Annexes and C are presented ecotoxicological and toxicological end-points of the pesticides that are more commonly used in the study area, in order to evaluate its contamination potential for surface waters based in Mackay´s fugacity model and pesticides risk classification on different ecosystems, EPRIP.

4.1.3. Predictive approaches for the environmental impact assessment of the selected pesticides

4.1.3.1. Physical-chemical and partition properties characterisation

Certain attributes of chemicals in the environment can be measured directly, particularly its concentrations. Other attributes cannot be measured directly, such evaporation rates, persistence and distance travelled. They can only be estimated by using models (OECD, 2001).

The physical-chemical properties and of environmental partition considered most important and at the same time, have a considerable influence on the environmental performance of pesticides are listed below.

a) Water solubility

Water solubility (S) quantifies the affinity of a substance for the water compartment, i.e., describes the maximum quantity of a substance which dissolves in a specific quantity of pure water, at a certain temperature, usually between 20 and 25ºC.

The higher the water solubility value, the more soluble the chemical and the probability of being transported by runoff to the surface waters, or to leach, from the soil to groundwater is superior (Waldron, 1997).

Although several pesticides do not leach because they are adsorbed on the soil particles or organic matter, they may still have a relatively high solubility (Waldron, 1997). So, in a first approach, the affinity for the water compartment can be quantified by the substance solubility (Vighi & Di Guardo, 1995).

Water solubility is through to be a key chemical property that affects the extent of both sorption and bioconcentration.

b) Vapor pressure

Vapor pressure (P) is the pressure exerted by the vapor of a substance in equilibrium with its pure phase (liquid or solid) at a given temperature. This represents the volatility, and therefore, the affinity for the air compartment, in its pure phase.

Some pesticides, such as fumigants, must be volatile in order to move and provide uniform distribution through the soil profile (Waldron, 1997).

Vapor pressure higher than 1 Pa generally indicates high volatility, whereas, below 10^{-6} Pa, the air affinity is very low. In general, intermediate values are highly influenced by other physical and chemical properties (Vighi & Di Guardo, 1995).

c) Henry's law constant and air-water partition coefficient

Henry´s law constant (H), usually expressed in Pa m^3/mol, represents the ratio between vapor pressure and water solubility. In practice, H represents a partition coefficient between air and water.

As losses of pesticides by volatilization depend on the partition of the substance between the gas and water, the K_{aw} and H can be taken as indices of affinity for media air (Bacci, 1994; Mackay *et al.,* 1997; Vighi & Di Guardo, 1995)**.**

Henry´s constant usually ranges between 10^{-9} and 10^{5} Pa m^3mol^{-1}, but for only some cases, pesticides show values higher than 10. Values higher than 10 Pa m^3mol^{-1} are always indices of very high air affinity.

d) Half -life and persistence

Persistence translates the pesticides degradation resistance. Persistence is routinely expressed as a half-life of the compound (DT_{50}). Half-life can be defined as the time required for half of the applied pesticide to be completely degraded, or broken down (Waldron, 1997).

The pesticide is subject to various degradation processes such as the reactions of hydrolysis, oxidation-reduction, photolysis and biodegradation. The persistence of the pesticide is dependent on these processes of degradation and the constant speed of degradation of reactions, ranging therefore, with pesticide intrinsic characteristics and the environmental compartment considered (Mackay *et al.,* 1997).

The half-life of the substance is affected by factors as the temperature, luminosity intensity and nature of microbial community so that there is not an exact and unique half- life (Mackay *et al.,* 1997).

In that way the persistence of the pesticide influences the potential for contamination, for example, the longer the compound lasts before it is broken down. The longer it is subject

to the forces of leaching, by this way, the degradation affects the potential for a pesticide to surface water (Waldron, 1997).

e) Organic Carbon Sorption Coefficient

This coefficient (K_{oc}) is assumed as an index of soil affinity and represents the sorption coefficient for the organic carbon of the soil. This coefficient is strictly related to octanol/water partition coefficient (K_{ow}).

It is an important parameter in the environmental evaluation of bioaccumulation in plants from air. In general values below 4 represents very low affinity for plants and values above 8 indicates high bioaccumulation potential (Vighi & Di Guardo, 1995).

f) Octanol/ water partition coefficient

This coefficient (K_{ow}) quantifies the lipophilicity of a substance (Vighi & Di Guardo, 1995) and can be defined as the ratio between the concentration of the chemical, on equilibrium, in the phase of octanol (C_o) and in the aqueous phase (C_w). Values of K_{ow} are expressed, usually in the form logarithmic (log K_{ow}).

It is used to estimate the ability of that substance to cross the biological membranes and to bioaccmulate in the organisms, i.e., as a measure of its biota affinity (Vighi & Di Guardo, 1995).

4.1.3.2 Mackay fugacity model - Level I

The environmental exposure assessment was based on Mackay´s fugacity model – Level I, which allowed the determination of pesticides affinity to the different environmental partitions, particularly to the water compartment.

There are two basic goals in pollutant modelling: to explain how a pollutant got where it is (a form of thermodynamic equilibrium) and to predict how fast a pollutant will move through an environmental compartment in the future (a form of kinetics) (Reemtsma *et al*., 2002).

It is well established that certain chemicals, when discharged to the environment, can persist for a sufficiently long period of time (months and years), can travel considerable distances (1000s of km) and can migrate between the available medium of air, fresh and marine waters, soils, sediments, vegetation and other biota, including humans. The environment is complex in nature and is continually changing, thus chemical fate is difficult to understand. Consequently, it is impossible to describe, or even know, the fate of chemicals accurately, but it is believed that the broad features of chemical fate can be understood and

even predicted, provided that sufficient information is available on certain key chemical and environmental properties (OECD, 2001).

Fundamental physical-chemical properties of a substance are used to quantify a chemical's behaviour in an evaluative environment; notable among these properties are partitioning properties (which control how the chemical is distributed at equilibrium between media, such as air and water and reactive properties, that govern how fast the chemical reacts or degrades (usually expressed for convenience as a half-life in each environmental medium) (Batista, 2003).

Chemical fate can be understood and even predicted when physical-chemical properties are combined with a multimedia model (OECD, 2001). So the knowledge of physical and chemical properties, including environmental partition of pesticides is essential to develop the predictive ability of its environmental impact before its introduction in environment (Batista, 2003).

An essential point is that these properties vary enormously in magnitude from chemical to chemical, i.e. by a factor of a million or more, thus chemical behaviour is correspondingly different for each factor. Environmental conditions such as temperature, sunlight intensity, rainfall and soil and vegetation types also vary greatly. Plus, the pesticides fate in surface waters is not determined by a single property of the pesticide but by a combination of properties (Waldron, 1997).

In fact, many models were used for the distribution estimative and levels of exposure of chemicals substances in different environment compartments, i.e., evaluate the predicted environment distribution (PED – Predicted Environmental Distribution) (Mackay *et al.,* 1997).

The Mackay's and its co-authors's fugacity model (Mackay, 1979, 1991, 1994; Mackay & Paterson, 1981; Mackay *et al.,*1997; Paterson & Mackay, 1985) is, among others, the most popular one.

The concept of evaluative model was introduced by Baughman & Lassiter (1978) for the prediction of the environmental distribution of chemical substances, purposely for the development a quantitative approach to the exposure evaluation. The evaluative model considerer an unspecified environment, based in standard universe properties. These models types are simple and easily and to handle, if we considerer that its require few input data (Mackay,1994; Vighi, 1993; Vighi & Di Guardo, 1995).

Fugacity is a criterion of equilibrium and is essentially a partial pressure (measured in Pa) and it is assumed to be proportional to concentration.

The fugacity model can be applied at different complexity levels - Level I to IV. Specifically for this work, the simplest level was considered (Level I – "Version 3.00, Trent University, Canada"), which translates the relative equilibrium partitioning of a conserved (i.e.

non-reacting) chemical in a multimedia setting, assuming the equilibrium and steady-state in a closed system, allows the PED's calculations of substances to various environmental compartments, for example, water, soil, air and sediment compartment.

Pesticides PED for the registered pesticides in Portugal for the most representative cultures in the study region was based in the Mackay Fugacity Model level I, using its last version (version 3.00, 2004, Trent University, Canada, obtained in http://www.trentu.ca/cemc/VBL1.html).

The application of this model allows the evaluation of environmental distribution and final faith of pesticides that with toxicological data, contribute for hazard evaluation of these products (Cerejeira, 1993).

A Level I model combines chemical partitioning (measured or estimated) data to give the Z values in each environment medium and, more importantly, the chemical's partitioning tendency. In this model, environment has no mechanisms for chemical to be added or removed and there are no degradation or advection processes. There is no active transport between environmental media; in fugacity terms, this assumption of equilibrium means that a single fugacity exists in the environment, i.e., in a four-compartment environment (OECD, 2001, Mackay *et al*., 1997).

For this model different data-bases are required, depending on the type of substance in consideration. The substances are classified, considering their water solubility (Sw) and vapor pressure (P), in 1, 2, 3 and 4 types.

Accordingly to this assumptions, the chemicals that are distributed for all media are considered type 1 (with water solubility superior to 10^{-6} mg/L and vapor pressure superior to 10-7 Pa); the type 2 corresponds to involatile chemicals (with water solubility superior to 10-6 mg/L and vapor pressure inferior to 10^{-7} Pa); the chemicals with zero, or near-zero solubility are type 3 (with water solubility inferior to 10^{-6} mg/L and vapor pressure superior to 10-7 Pa) and finally the chemicals practically insoluble and involatile, for which the Mackay Model is not applied are type 4 (Mackay *et al.,*1996; Trent University, 2004).

Considering the Molar Mass, Temperature (20ºC in the simulation), Water solubility, Vapour Pressure, log $_{KOW}$ and Melting Point values, pesticides PED, which experimented partition in all the environmental compartments considered (water, soil, sediments, suspended solids and fish), can be consulted in the Annex D. In discussion, the principal results are represented considering the pesticides with increased affinity to water.

4.1.3.3 Rating systems for pesticide risk classification on different ecosystems

The basic long-term challenge for agriculture is to produce food and industrial crops efficiently, profitably and safely, and to meet a growing world demand without degrading natural resources and the environment (Hond *et al.,* 2003).

Nowadays, registration procedures in many countries (i.e., EU) require the evaluation of all potential risks of environmental damage that might be associated with the use of plants' protection products (Finizio *et al*., 2000).

In order to respond to the challenge and develop better policies, policymakers need agri-environmental indicators, which can help monitor the environmental effects of agriculture and provide a tool for policy analysis.

Pesticide indicators can provide a useful tool for the domestic policies and international obligations evaluation, related to pesticides use in agricultures, and can also convey a general idea about pesticide use, risk management and the pesticides impact on human health and environment (Hond *et al.,* 2003).

Different strategies for risk management have been proposed in the last few years with different targets; however, in the present days, the criteria used to decide the acceptance of environmental risks are generally based on the concept of toxicity/exposure ratio (TER) (Finizio *et al.*, 2000).

The EPRIP index, is according to Finizio *et al.* (2000), the result of sponsored project ANPA (Agenzia Nazionale Protezione Ambiente of Italy) for setting up different rating indexes for pesticides for different environmental scenarios.

On one hand, a TER (Toxicity Exposure Ratio) is the ratio between a toxicological end- point (i.e., LD_{50}, NOEL) and PEC (Predicted Environmental Concentration). This ratio should be calculated for each of the environmental compartments at risk (ground water, surface water, soil) to establish critical thresholds as a trigger for the need of further information.

On the other hand, TERs can be used for making comparisons with appropriate "safety factors" representing the acceptable limit of risk for the different components of the environment (Finizio *et al.*, 2000).

Risk indicators selected for this study were based up the consideration of three different environments (surface waters, terrestrial hypogean, and epygean systems) in a worst-case scenario context. For each of these systems, two different time-space scales were considered. The short term at local scale indexes, refers to a risk posed by a pesticide, immediately after application to the three different systems; and the long term at a wider area in a medium period.

The pesticides risk Index considered for this work are based on exposure indicators (rate of application, environmental distribution, bioaccumulation, and soil persistence) and on the effects (i.e., EC_{50}, NOEL) that these substances can exert on non-target organisms considered representative of the three environmental systems, according to Directive 414/91/EC (e.g., algae, *Daphnia*, fish for surface water).

The values of the toxicological endpoints and physical–chemical properties used in this classification method have been compiled by adequate literature, particularly in Tomlin (2006) and completed within the possible through reliable databases available on the Internet (FOOTPRINT PPDB), and also used predicted environmental distribution values resulted from the Mackay fugacity model.

One of the main limitations is due to the fact that information required for the calculation of some pesticide risk Index was not always available. Consequently, when literature data was not available, default or estimated values have been applied (Table 4.2). Obviously, due to the high number of parameters involved in the characterization of environmental risk and the impossibility of producing quantitative values for either exposure or effects, we have to considerer the possibility of indicators over evaluation.

Table 4.2 - *Default or estimated values considered for the calc of Indexes*

INDEXES	DEFAULT OR ESTIMATED VALUES
PRIHS-1	No or very few data were available on beneficial arthropods; as a default, the same score given to bees in PRIES-1 was used.
PRIHS-2	No or very few data are available on beneficial arthropods; as a default, the same score given to bees in PRIES-1 was used.
PRIES-1	No data are available for beneficial arthropods and plants: for beneficial arthropods, the same default in PRIHS-1 was assigned.
PRIES-2	For plants the lowest score (0.1) was assigned to insecticides and fungicides, the highest (4) to herbicides; no or very few data were available on beneficial arthropods; as a default, the same score given to bees in PRIES-1 was used.

PRIHS-1: Short-Term Pesticide risk index for the hypogean soil system.

PRIHS-2: Long-Term Pesticide risk index for the hypogean soil system.

PRIES-1: Short-Term Pesticide risk index for the epygean soil system.

PRIES-2: Long-Term Pesticide risk index for the epygean soil system.

Short-Term Pesticide Risk Index for the Hypogean Soil System (PRIHS-1)

This index calculates the risk for non-target hypogean organisms immediately after a pesticide application. PEC was calculated assuming that the product spreads uniformly on a

surface of 1ha and on a layer of 5 cm. Assuming the soil density was equal to 1.5 g/cm^3, the PEC was calculated as:

$$PEC=MRA/750 \quad (eq.1)$$

where MRA=maximum rate of application (g/ha), and 750 = 10 000 m^2 x 5 cm x 1.5 g/cm3 x 750,000 kg. As the PEC is expressed as milligrams per kilogram of soil, this value is corrected by a factor of 1000.

According to the Uniform Principles, earthworms, beneficial arthropods and mammals have been selected as non-target organisms representative of the soil system. Scores and weights assigned to the different intervals of categories in which the possible TER values have been subdivided are presented in table 4.3. According to the test method proposed by EPPO (1994a), a real TER cannot be calculated for beneficial arthropods.

Therefore, the score was assigned in function of the observed effect for the three exposure levels. The final score of the chemical, ranging from 0 to 100, was calculated by means of the following algorithm [2]:

$$\textbf{PRIHS-1= (A x 5.5) + (B x 5) + (C x 2)} \quad \textbf{(eq.2)}$$

Table 4.3 - *Categories with relative scores and weight for non-target organisms representative of the hypogean soil system (adapted from Finizio et al., 2000).*

EARTHWORMS (A)		BENEFICIAL ARTHROPODS (B)		MAMMALS (C)	
(EC_{50}/PEC)	Score	% Effect (MRA)	Score	(LD_{50derm}/PEC)	Score
>1000	0	(2 x MRA) = 0%	0	>1000	0
1000 -100	1	0% < MRA < 30%	2	1000 -100	1
100-10	2	MRA> 30%	4	100-10	2
10-1	4	(0.5 X MRA) > 30%	8	10-1	4
<1	8			<1	8
W = 5.5		W = 5		W = 2	

EC_{50} – median effective concentration; LD_{50der} – median lethal dose (by dermal contact);
MRA – maximum application rate; PEC – predicted environmental concentration

Long-Term Pesticide Risk Index for the Hypogean Soil System (PRIHS-2)

For this index, application period time and persistence of the substance was considered. Consequently a time-weighted average PEC was calculated as

$$\textbf{PEC}_{LT} = \textbf{PEC}_{ST}(1-e^{-kt})/kt \quad \textbf{(eq.3)}$$

where PEC_{LT} = predicted environmental concentration in soil after a given time;

PEC_{ST} = predicted environmental concentration immediately after the application (cf. previous index); *t* = period time considered in function of the toxicological test (i.e., 14 days for earthworms, 730 days for mammals); and $k = \ln 2/DT_{50}$.

Microorganisms, not considered in the short-term index, have been included, assuming that their role is higher in the long run. As for arthropods, the expression of test results does not allow the calculation of a real TER.

Also, the exposure via contaminated food for the mammals has been considered. In this case a diet concentration (DC: mg/kg), expressed as the product of the bioconcentration factor (BCF) and the PEC_{LT}, has been calculated. Table 4.4 reports the scores and weights assigned to the different intervals of categories in which the possible TER values (or effect levels) have been subdivided. The final score of the chemical can be obtained by means of the following algorithm [4]:

$$\mathbf{PRIHS\text{-}2 = (A \times 4) + (B \times 4) + (C \times 3)(D \times 1.5)} \quad \mathbf{(eq.4)}$$

Table 4.4 - *TER Categories with Relative Scores and Weight for Nontarget Organisms Representative of the Hypogean Soil System (adapted from Finizio et al., 2000).*

Earthworms (A)		Microorganisms (B)		Beneficial arthropods (B)		Mammals (C)	
(LC_{50}/PEC) (14 days)	Score	% Effect	Score	% Effect	Score	(NOEL/DC) (2 years)	Score
>1000	0	(2 x MRA) = 0%	0	(2 x MRA) = 0%	0	>1000	0
1000 -100	1	0% <MRA <25%	2	0% <MRA <30%	2	1000 -100	1
100-10	2	MRA> 25%	4	MRA> 30%	4	100-10	2
10-1	4	(0.5 X MRA)> 25%	8	(0.5 X MRA)> 30%	8	10-1	4
<1	8					<1	8
W = 4		**W = 4**		**W = 3**		**W = 1.5**	

EC_{50} – median effective concentration; PEC – predicted environmental concentration; MRA – maximum application rate; NOEL – no-observed-effect-level.

Short-Term Pesticide Risk Index for the Epygean Soil System (PRIES-1)

This index evaluates the risk for epygean non-target organisms immediately after a pesticide application. For bees, the score applied was based in the hazard quotient (HQ), i.e., the ratio between the MRA (maximum rate of application) (g/ha) and the LD_{50} (µg/bee). For mammals the score has been calculated as a ratio between the LD_{50} (mg/kg) and the TDI (total daily intake) - (mg/kg) - also identified as ADI (acceptable daily intake) (Health Canada, 1981).

The table 4.5 was the base to obtain the necessary scores and weights to calculate the final score according to the next algorithm [5]

$$PRIES\text{-}1 = (A \times 3) + (B \times 4) + (C \times 3) + (D \times 2.5) \quad (eq.5)$$

Table 4.5 - *Risk Classification Intervals, Scores, and Weight for Epygean Non-target Organisms (adapted from Finizio et al., 2000).*

Bees (A)		Birds (B)		Beneficial arthropods (C)		Mammals (C)	
HQ	Score	(LD_{50}/ADI)	Score	% Effect	Score	(LD_{50}/ADI)	Score
<1	0	>1000	0	(2 x MRA) = 0%	0	>1000	0
1-10	1	1000-100	1	0% <MRA <30%	2	1000 -100	1
10-100	2	100-10	2	MRA> 30%	4	100-10	2
100-1000	4	10-1	4	(0.5 X MRA)> 30%	8	10-1	4
>1000	8	<1	8			<1	8
W = 3		W = 4		W = 3		W = 2,5	

HQ – hazard coefficient; LD_{50} – median lethal dose; MRA – maximum application rate; ADI – acceptable daily intake.

Long-Term Pesticide Risk Index for the Epygean Soil System (PRIES-2)

With regard to the variability of possible environmental scenarios, a PEC cannot be calculated; consequently this index is qualitative due to the impossibility of obtaining a quantitative TER.

Besides application rate, exposure parameters include persistence expressed as DT_{50} in soil; bioconcentration potential expressed as log K_{ow}; and affinity for the soil and air compartment expressed as percent and distribution calculated by means of the standard Fugacity Level I model.

For the PRIES-2 index calculation, weights and scores of tables 4.6 and 4.7 have been used, as well as the next algorithm [6].

$$PRIES\text{-}2 = \frac{\sum_{i=1}^{5} Ti}{5} \times \frac{(A+S)}{2} \times B \times P \times MRA \quad (eq.6)$$

Table 4.6 - *Scores Assigned to the Exposure Parameters (adapted from Finizio et al., 2000).*

Persistence (P)		Bioaccumulation (B)		Air affinity (A) Fugacity Level I		Soil affinity (A) Fugacity Level I		Application rate (MRA)	
Dt_{50} (d)	Score	($logK_{ow}$)	Score	%	Score	%	Score	g/ha	Score
<10	1	<2.5	1	<0.01	1	<1	1	<50	1
10-30	2	2.5-3.5	1.1	0.01-5	1.25	1-20	1.25	50-200	2
30-90	3	>3.5	1.25	>5	1.5	>20	1.5	200-1000	3
90-300	4							1000-10000	4
>300	5							>10000	5

DT_{50} – half-life; log Kow – logarithm of the partition coefficient octanol/water;
MRA – maximum application rate

Table 4.7 - *Scores Assigned to the Effect Parameters (adapted from Finizio et al., 2000).*

Plants (T1)		Bees (T2)		Birds (T4)		Mammals (T5)	
FITOT.	Score	LD_{50} (µ/bee)	Score	LD_{50} (mg/Kg)	Score	NOEL (mg/Kg)	Score
+	4	<0.1	4	<0.1	4	<0.1	4
-	0.1	0.1-1	3	0.1-1	3	0.1-1	3
		1-10	2	1-10	2	1-10	2
		10-100	1	10-100	1	10-100	1
		>100	0.1	>100	0.1	>100	0.1

LD_{50} – median lethal dose; NOEL – no-observed-effect-level.

Short-Term Pesticide Risk Index for the Surface Water System (PRISW-1)

This index evaluates the risk occurring immediately after pesticide application in a surface water system (1-m depth) adjacent (20 m) to the treated area. The PEC is obtained by the sum of Q_D (rate of pesticide reaching the water body by drift) and R_o (runoff). Drift is calculated by

$$\mathbf{Q_D = MRA \times D_F \quad (eq.7)}$$

where Q_D = rate of pesticide reaching the water body by drift; MRA = maximum rate of application; and D_F=drift fraction (assumed to be 4% according to Finizio *et al.,*2000).

Pesticide concentration in the water body (PEC_{st}), as already stated, is obtained by the sum of Q_D and R_o. However, R_o value was not calculated. So, as a default the PEC_{st} value was based in the % H_2O (PED) calculated with Fugacity Level I, presented in table 3.9. So, to calculate the index the weights and scores of tables 4.7 and 4.8 have been used. The final score was obtained by application of the following the logarithm [8]:

$$\mathbf{PRISW\text{-}1 = (\mathit{A} \times 3) + (\mathit{B} \times 4) + (\mathit{C} \times 5.5) \quad (eq.8)}$$

Table 4.8 - *Risk Classification Intervals, Scores, and Weight for Nontarget Organisms in Surface Water System (adapted from Finizio et al., 2000).*

ALGAE (A)		*Daphnia magna*(B)		FISH (C)	
(EC_{50}/PEC)	Score	(EC_{50}/PEC)	Score	(LC_{50}/PEC)	Score
>10000	*0*	*>10000*	*0*	*>10000*	*0*
10000-1000	*1*	*10000-1000*	*1*	*10000-1000*	*1*
1000-100	*2*	*1000-100*	*2*	*1000-100*	*2*
100-10	*4*	*100-10*	*4*	*100-10*	*4*
10-2	*6*	*10-2*	*6*	*10-2*	*6*
<2	*8*	*<2*	*8*	*<2*	*8*
W= 3		***W= 4***		***W= 5.5***	

LD_{50} – median lethal dose; EC_{50} – median effective concentration;
PEC – predicted environmental concentration

Table 4.9 - *Classes of Concentrations in function of the relationship between the percentage of water distribution (Fugacity Level I) and the PEC_{ST} Obtained Using SoilFug Model (adapted from Finizio et al., 2000).*

$\%H_2O$ (Fugacity level I)	PEC_{ST} (SoilFug)(mg/L)	DT_{50} soil (d)	SCORE
>95	1.0E-02-**1.0E-01**	<5	0.01
60-95	1.0E-03-**1.0E-02**	5-10	0.1
20-60	1.0E-04-**1.0E-03**	10-30	1
2-20	1.0E-05-**1.0E-04**	30-90	10
0.1-2	1.0E-06-**1.0E-05**	90-300	50
		>300	100

PEC_{st} - Pesticide concentration in the water body; DT_{50} – half-life period in soil

Long-Term Pesticide Risk Index for the Surface Water System (PRISW-2)

It is possible to define six different classes of water concentration (CCW) if we consider the upper limits of the intervals of PEC_{ST} (worst-case scenario) (CCW: bold in table 4.9). A theoretical concentration in water (TCW) was calculated, multiplying CCW by MRA and dividing by a factor of 10, assumed as a dilution factor in the receiving water body at the mean scale [9]:

$$\textbf{TCW (mg/L) = (MRA x CCW)/10} \quad \textbf{(eq.9)}$$

The theoretical exposure in water (TEW: mg/L) was obtained multiplying TCW by the score for persistence. Finally, TERs is the ratio between the NOEL for aquatic organisms and

the TEW. The final score, ranging from 0 to 100, using the scores and weights of tables 4.9 and 4.10, was calculated by

$$\text{PRISW-2} = \sum(TER \times W) \times B \times S \quad \textbf{(eq.10)}$$

where *B* and *S* refer to the scores of the bioaccumulation potential (logK_{ow}) of the substance and its percentage distribution in sediments (Fugacity Level I).

Table 4.10 - *TER Classification, Score, and Weight for Nontarget Organisms Representative of Surface Water System (adapted from Finizio et al., 2000).*

ALGAE		DAPHNIA		FISH		BIOACCUMULATION		SEDIMENT AFFINITY FUG. LEVEL I	
TER	SCORE	TER	SCORE	TER	SCORE	log K_{ow}	score (B)	%	Score (S)
>1000	*0*	*>1000*	*0*	*>1000*	*0*	*≤ 2.5*	*1*	*<1*	*1*
1000-100	*1*	*1000-100*	*1*	*1000- 100*	*1*	*2.5 –3.5*	*1.1*	*1-30*	*1.1*
100 – 10	*2*	*100 – 10*	*2*	*100-10*	*2*	*>3.5*	*1.25*	*>30*	*1.25*
10 – 1	*4*	*10 – 1*	*4*	*10 – 1*	*4*				
<1	*8*	*<1*	*8*	*<1*	*8*				
W = 2		*W = 3*		*W = 3*					

TER - Toxicity Exposure Ratio; log K_{ow} – logarithm of the partition coefficient octanol/water

Environmental Risk Index for Pesticides (ERIP)

ERIP represents the environment pesticides overall risk assessment. For this toxicity index, representative organisms of main levels of taxonomic and ecological organization for the three environmental typologies (aquatic, terrestrial epygean, terrestrial hypogean) have been chosen. Exposure parameters include % environmental compartment distribution (air, soil, water, and sediment) determined by Fugacity Level I; persistence, bioaccumulation potential, and MRA (Table 4.11).

Toxicological values have been defined as the mean of scores assigned to pesticides toxicity on selected organisms, expressed as T_{WAT} (toxic substance effect in water), T_{EPY} (toxic substance effect in non-target terrestrial epygean organisms) and T_{HYPO} (toxic substance effect in non-target terrestrial hypogean organisms) for different organisms, represented in tables 4.13 to 4.15.

In many cases, both acute and chronic toxicity were used in function of the availability of data. The toxic effects (T_x) was based in eq.11:

$$T_X = \frac{(\sum_1^n \text{scores})}{n} \quad \textbf{(eq.11)}$$

where T_x= average score for the substance toxic effects in a particular environmental system; and *n* = numbers of individual toxicity scores utilized.´

Thus, considering different weights (W_i) (1.5 for the system most at risk and 0.5 for the other two systems), D *values* (exposure parameters) and T values (effect parameters) the index was based in equation [12] (using tables 4.11 to 4.15),

$$\mathbf{ERIP = [(D_{[(W+SED)/2]} x\ T_{WAT})x\ W_1 + (\ D_{[(A+S)/2]} x\ T_{EPY}\ x\ W_2 + (D_S\ x\ T_{HYPO})\ x\ W_3]\ x\ P\ x\ B\ x\ MRA \quad (eq.12)}$$

where $D_{[(W+SED)/2]}$ = mean of the scores assigned to the percentage of chemical distribution in water and sediments (Fugacity Level I); $D_{[(A+S)/2]}$= mean of the scores assigned to the percentage of chemical distribution in air and soil (Fugacity Level I); D_S = mean of the scores assigned to the percentage of chemical distribution in soil (Fugacity Level I); T_{WAT} , T_{EPY} , T_{HYPO} = average scores for effects in water epygean and hypogean soil systems; W=weights; *P*= score for persistence; *B*= score for the potential bioaccumulation; and MRA= score at the maximum rate of application.

Table 4.11 - *Air, Water, Soil, and Sediment classes' affinity for pesticides and relative Scores and weights (adapted from Finizio et al., 2000).*

Air affinity Fugacity Level I (DA)		Water affinity Fugacity Level I (DW)		Soil affinity Fugacity Level I (DS)		Sediment affinity Fugacity Level I (DS_{ED})	
%	Score	%	Score	%	Score	%	Score
<0.1	0.5	<0.1	0.5	<0.1	0.5	<0.1	0.5
0.1 – 1	1	1 – 10	1	0.1 – 5	1	0.1 – 5	1
1-5	1.25	10-50	1.25	5-10	1.25	5-10	1.25
5 – 20	1.5	50 – 90	1.5	10– 30	1.5	10 – 30	1.5
>20	2	>90	2	>30	2	>30	2
W = 1		**W = 1.5**		**W = 1**		**W = 0.5**	

Table 4.12 - *Risk Classification Intervals, Scores, and Weight for Persistence, Bioaccumulation, and Rate of Application of Pesticides (adapted from Finizio et al., 2000).*

Persistence (P)		Bioaccumulation (B)		Max. rate of application (MRA)	
DT_{50} (d)	Score	(log K_{ow})	Score	(g/ha)	Score
<10	0.5	< 2.5	1	< 50	0.5
10-13	1	2.5 – 3.5	1.1	50 - 200	1
30 - 90	2	>3.5	1.25	200 - 1000	2
90 - 300	3			1000 – 10000	3
>300	4			>10000	4

DT_{50} – half-life; log Kow – logarithm of the partition coefficient octanol/water

Table 4.13 - *Long and short term toxicity classification and their relative scores for Algae, Daphnia and fish (adapted from Finizio et al., 2000).*

ALGAE			*DAPHNIA*			FISH		
(NOEC) (96 h)	EC_{50} (96h) (mg/L)	Score	(NOEC) (21-28 d)	EC_{50} (48h) (mg/L)	Score	(NOEC) (14-28 d)	EC_{50} (96h) (mg/L)	Score
<0.01	<1	2	$<10E^{-3}$	<0.1	2	$<10E^{-3}$	<0.1	2
0.01 - 0.1	1 – 10	1.5	$10E^{-3}$- $10E^{-2}$	0.1 – 1	1.5	$10E^{-3}$- $10E^{-2}$	0.1 – 1	1.5
0.1 - 1	10 – 100	1	$10E^{-2}$ –$10E^{-1}$	1 – 10	1	$10E^{-2}$ –$10E^{-1}$	1 – 10	1
1-10	100- 1000	0.5	$10E^{-1}$ - 1	10 – 100	0.5	$10E^{-1}$ - 1	10 – 100	0.5
>10	>1000	0.1	>1	>100	0.1	>1	>100	0.1

EC_{50} – median effective concentration; NOEC –no-observed-effect.concentration

Table 4.14 - *Toxicity pesticide classification and relative scores on terrestrial Epygean non-target organisms (adapted from Finizio et al., 2000).*

Plants		Bees			Ben. arthropods				Birds(D) and Mammals(E)
Phyt	Score (A)	NOEL (µg/bee)	LD_{50} (µg/bee)	Score (B)	%	Score (C)	NOEL (mg/kg	LD_{50} (mg/kg)	Scores (D) and (E)
+	2	<0.01	<0.1	2	>80	2	<1	<10	2
-	0.1	0.01-0.1	0.1-1	1.5	80-50	1.5	1-10	10-10E2	1.5
		0.1-1	1-10	1	50-30	1	10-10E2	10E2-10E3	1
		1-10	10-100	0.5	30-10	0.5	10E2-10E3	10E3-10E4	0.5
		>10	>100	0.1	<10	0.1	>10E4	>10E4	0.1

LD_{50} – median lethal dose; NOEL – no-observed-effect-level

Table 4.15 - *Classification of pesticide toxicity and relative scores on terrestrial Hypogean nontarget organisms (adapted from Finizio et al., 2000).*

EARTHWORMS			MICROORGANISMS	
NOEL (mg/kg d)	LD_{50}	Score (A)	% effect	Score (B)
<0.1	<1	2	(0.5 x MRA)>25%	2
0.1 -1	1-10	1.5	MRA>25%	1.5
1-10	10 -10E2	1	0%<MRA<25%	1
10 -100	10E2 -10E3	0.5	(2 x MRA)= 0%	0.1
>100	>10E3	0.1		

NOEL – no-observed-effect-level; MRA – maximum application rate

4.1.4. Surface waters and sediments sampling in the study area

In the area of study, it was selected four sampling dates in order to evaluate the surface waters exposure to pesticides and its dynamic - June 5th, June 27th, July 22nd and August 13th. This involves different pesticides application and irrigation periods.

The surface water samples were collected in Almonda River, specifically, upstream (AlmondaR-1) and downstream (AlmondaR-2) of the RNPB, as well as in "Alverca do Campo".

Thus, there was a total amount of 14 surface water samples for all the different areas (Table 4.6).

The water samples resulted from a simple sampling and were collected into 1L glass vessels (Figure 4.6).

The sediments were collected only on August 13th at "Alverca do Campo" (D_{20}-1) once it would be the closest date to the execution of the toxicity evaluation tests (using the larvae of freshwater Midges - *Chironomus riparius)* and due to accessibility issues. The sediments samples were collected at a depth of 1cm and into dark vessels (Figure 4.6).

All the samples (of water and sediments), properly identified with the location's name and sampling date, were taken into the laboratory in refrigeration conditions in order to proceed with the tests (Figure 4.6).

At the laboratory, water samples were stored in the dark and kept at 4 ºC until analysis. All the samples were filtered with 0.45 µm glass fiber filters in order to determine the true dissolved chemical concentration.

Table 4.16 - *Location and dates of surface and sediment samples in 2008.*

		Date							
		June 5th		June 27th		July 22nd		August 13th	
Ponto de amostragem		Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
Almonda River	AlmondaR -1	√	×	√	×	√	×	√	×
	AlmondaR -2	√	×	√	×	√	×	√	×
Alverca do Campo	D_{20-1}	√	×	√	×	√	×	√	√
	D_{20-2}	×	×	√	×	√	×	×	×

√ **sampling**
× **No sampling**

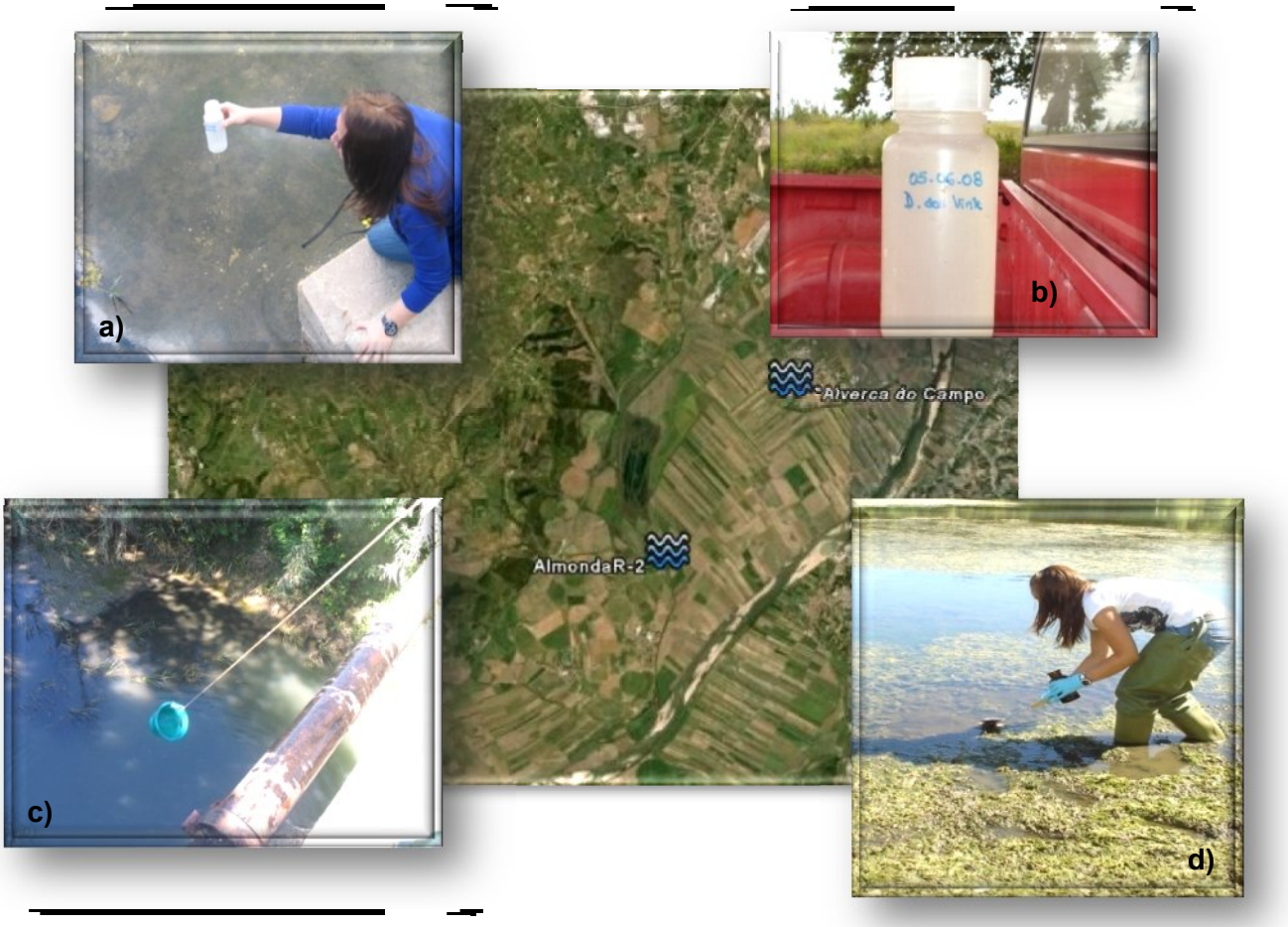

Figure 4.6 - *Water and sediment sampling for the different areas:*
a) D_{20}-2 sampling, b) D_{20}-1 sample, c) AlmondaR-2 sampling and d) D_{20}-1 sediment sampling.

4.1.5 Analytical methodology to surface water exposure assessment to pesticides – solid phase microextraction (SPME) and gas chromatography coupled to mass spectrometry (GC-MS)

A number of toxic compounds have been designated priority pollutants [e.g., those on lists of the US Environmental Protection Agency (EPA) and the Water Framework Directive of the European Union (EU)] and their measurement is necessary to ensure that water quality standards are maintained (Vrana *et al.,* 2005).

Sampling and analysis of such a broad range of organic (e.g., chlorophenols, organo-chlorine pesticides, polyaromatic hydrocarbons, polychlorinated biphenyls) and inorganic (e.g., heavy metals and some of their organo-metallic species) compounds represents an ongoing challenge at present (Vrana *et al.,* 2005; Barceló, 2000).

Until now, about 400 articles on SPME have been published in different fields, including environment (water, soil and air), food, natural products, pharmaceuticals, biology, toxicology, forensics and theory (Alpendurada, 2000).

Solid-phase microextraction (SPME) is a relatively recent technique, which was introduced in the early 90's by Pawliszyn and Lord. Although as part of sorbents extraction methods, it is a solvent-free one (Dietz *et al.,* 2006; Eisert *& Levsen,* 1996).

In the last years, solid-phase microextraction (SPME) has reached a widespread acceptance simultaneously for analyte matrix separation and preconcentration, and it has been applied, in a large scale, for environmental samples, food and pharmaceuticals (Dietz *et al.,* 2006; Ouyong *&* Pawliszyn*,* 2006).

This technique consists on establishing a balance between the chemical substance and a fused silica fibre coated with a stationary phase, which can be a liquid polymer, a solid sorbent, or a combination of both. After adsorption, when the coated fibre is exposed to the sample for a given period of time, the fibre is introduced into the heated injector of the gas chromatograph and exposed for a certain time, occurring the organic compounds desorption from the polymeric phase (Stashenko *et al.,* 2004; Dietz *et al.,* 2006; Eisert *et al.,* 1996).

Extraction conditions, such as: extraction period; sample's temperature and pH; the adding of soluble salts and the presence of organic solvents in the sample influence the affinity fibre's stationary phase analytes.

Also undoubtedly relevant, are the desorption conditions, being given special attention to the temperature and GC injector desorption period.

Presently, this technique continues to be headed towards GC, because this combination enables the minimization of any potential analyte losses due to multi-step processes (Stashenko *et al.,* 2004).

The popularity of GC is based on a favorable combination of very high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity. Indubitably, CG has contributed to the current analytical possibilities for the measurement of volatile organic compounds in the environment.

Plus, GC combined with MS (GC-MS) provides conclusive and defensible analytical information for the analysis of environmental samples containing organic compounds amenable to GC analysis (Santos & Galceran , 2002).

As the scope of SPME grew, new improvements were made with the appearance of new coatings that allowed an increase in the specificity of this extraction technique (Alpendurada, 2000).

As stated above, one of the main advantages of this technique is its solvent-free nature, which by itself represents benefits from both toxicological and environmental perspectives.

In a general way**,** SPME increasingly substitutes classical and time consuming extraction and leaching processes. Besides its simplicity, the SPME technique is sensible and precise; doesn't demand great amounts of water to extract the analytes, which facilitates the transportation and the storage field to the laboratory. It may be easily automated, and the apparatus is inexpensive (Vrana *et al.,* 2005; Barceló, 2000).

The procedure for the extraction of pesticides residues from water samples, by "Solid phase microextraction" **(**SPME) and dosage by "Gas chromatography-mass spectrometry" (GC-MS), performed in the Ecotoxicology Laboratory of DPPF/ISA, is expressed in the Annex E.

4.1.6 Bioassays to toxicity assessment on surface waters and sediments

Regardless of all the technological changes implemented to reduce toxicity the use of pesticides in the agriculture continues to often present toxicity to a range of groups of aquatic organisms.

The aquatic environment usually represents the final destination of contaminants from problematic areas, where they can affect local biota, directly or indirectly. Algae, crustaceans, insect larvae and fish are the most commonly used test species in aquatic ecotoxicology.

In order to fully evaluate the environmental impact of pesticides, both physical-chemical and toxicological analyses, should be performed (EPA, 2002).

Physicochemical analyses do not provide information about the environmental samples toxicity. Toxicity detection is crucial in assessing environmental contamination, especially if mixture compounds are suspected (Ruiz *et al.,* 1997; Boluda *et al.,* 2002; EPA, 2002).

Microbial tests have been widely used in toxicity screening because of the similarity of complex biochemical functions with higher organisms, ease of handling, short exposure time, and reproducibility of the interlaboratory results (Ruiz *et al.,* 1997; Boluda *et al.,* 2002).

To assess cause-and-effect relationships between pesticides and biological responses, a test battery was used with organisms that occupy key functions in the ecosystems, particularly *Vibrio fischeri* (bacteria; decomposer), *Pseudokirchneriella subcapitata* (planktonic microalgae; primary producer), *Daphnia magna* (planktonic

cladoceran; primary consumer; filter feeder) and *Chironomus riparius* (benthic midge larvae; deposit feeder).

The concept of a concentration-response, or more classically, a dose-response relationship is “the most fundamental and pervasive one in toxicology”. This concept assumes that there is a cause and effect relationship between the dose of a toxicant (or concentration for toxicants in solution) and a measured response (Ghosh, 1997).

Nevertheless, no single test method or test organism can be expected to satisfy a comprehensive approach to the environmental conservation and protection, but enables the management of the main problematic issues for the environment or even the definition of "safe" or "no effect" concentrations of substances that exist in surface waters (Environment Canada, 1992; EPA, 2002).

4.1.6.1 Growth inhibition tests using the freshwater alga *Pseudokirchneriella subcapitata*

Algae are the primary producers in the ecosystems. If they are affected by pesticides, the balance within the ecosystem could change (Hanazato, 2000; McCormick & Cairns, 1994).

The algae *Pseudokirchneriella subcapitata* (formerly nominated as *Raphidocelis subcapitata* and *Selenastrum capricornutum*) is part of the *Chlorophyceae* class, the *Chloroccoccales* order and the Scenedesmaceae family. *Pseudokirchneriella subcapitata* is an immobile unicellular green algae and with the length of 40 to 60 μm^3 (Environment Canada, 1992).

Growth inhibition tests with unicellular algae (e.g. *Chlorella*, *Chlamydomonas*, and *Pseudokirchneriella*) have long been used to evaluate the bioavailability and toxicity of surface water contaminants.

For both ecotoxicological and scientific matters it represents one of the most important groups of algal from freshwater, as it is a part of phytoplankton and extensively used in the laboratories (Amaral, 2004; Environment Canada, 1992).

A test substance is considered toxic when a statistically significant dose-dependent inhibition of algal growth occurs, i.e., the difference among the algal growth in an appropriated control and the growth of algal exposed to the sample is statistically significant, being the endpoint of this test the growth inhibition of the algae.

The acute toxicity tests were based upon the OECD (1984) and EEC (1989) guidelines (microplate technique) and the ISO/DIS 8692 (“Algaltoxkit F^{TM}”) respectively expressed in Annexes F and G for samples AlmondaR-1 and AlmondaR-2 as well as D_{20}-1 and D_{20}-2.

However, some authors' consider that this toxicity tests performed with the *P. subcapitata* can be designated as chronic tests or furthermore short-chronic tests. For this work, it was considered that the toxicity assessment for the algae belongs to the acute toxicity group based on the premise that acute toxicity tests are short-term tests designed to measure the effects of toxic agents on aquatic species during a short period of their life span, specifically effects over 24 to 96 hour period (Hoffman *et al*., 2003).

a) The microplate technique - OECD (1984) and EEC (1989) guidelines

The microplate technique is a scaled-down version of the standard USEPA algal bottle test, which enables a number of advantages over the standardized algal bottle tests, such as the utilization of small sample volumes, small volume of algae, and less space for the incubation (Miller *et al.,* 1978; USEPA, 1989; Environment Canada, 1992).

This technique can be used as a screening test increasing in this way the efficiency in the processing of samples, opposing to the classic algal bottle test (Environment Canada, 1992), and has been developed specifically for *P. subcapitata,* although it can also be used with other test species of algae (Environment Canada, 1992).

However, this procedure has some limitations, as for example the previous sample filtration might influence the toxicity, which can significantly cause a reduction. Other limitation is that for high concentrations of dissolved organic material the results can be ambiguous (Environmental Canada, 1992).

In accordance with the above mentioned both samples - AlmondaR-2 and D_{20}-1 (at August 13th) - were subjected to a concentration series of 6.25, 12.5, 25, 50 and 100% in both samples in order to assess the acute toxicity.

b) The "Algaltoxkit F^{TM}" test

The "Algaltoxkit F^{TM}" test when compared with the standardized algae bottle test, this one has more advantages since it does not require an algae culture medium, the microalgal are presented immobile on a special matrix designated as water pearls; allows a quick result and the absorbance measurement of the samples using a spectrophotometer.

In accordance with the above mentioned both samples were subjected to a concentration of 100% in order to assess the acute toxicity ("Algaltoxkit F^{TM}").

Both tests had a duration of 72-h of exposure and its growth allowed the count of cells with relative precision after 72 hours (Environment Canada, 1992).

The mean specific growth rate (GR) per day was estimated based on equation [13].

———— [13]

Where:

N_n= measured number of cells/mL at time t_n

N_1= measured number of cells/mL at time t_1

t_n =time of n^{th} measurement after beginning of test

t_1=time of first measurement after beginning of test

4.1.6.2. Biological test using the *Daphnia magna*

Since the 1940s, there are records of previous utilization of the *Daphnia magna* in toxicity tests by Anderson (1944). In the last 20 years it has been extensively used in regulatory testing as well in basic ecotoxicological research.

Daphnia magna Straus, commonly known as water flea, is a micro crustacean from the phylum *Arthropoda*, class *Crustacea*, subclass *Branchiopoda*, order *Cladocera* and family *Daphniidae.*

Widely distributed through the world, it is part of the zooplankton, primary consumer in the trophic chain and a filter feeder. *D. magna* is referred as “key invertebrate” to toxicity battery tests, especially when used in a preliminary phase of the risk evaluation process, and used by international organizations like USEPA and OECD (Barros, 2005).

D. magna lives in eutrophic small ponds and rockpools. Such environments are unpredictable, having wide fluctuations in pH value, temperature, oxygen concentration, salinity, and other non biotic factors. However, *D. magna* is capable of adapting to such conditions.

This species are recurrently used in bioassays, primarily for their sensitiveness to a broad range of aquatic contaminants, and secondly for their inner features, like their small size, fast testing due to their short life span, and for their relatively easiness of laboratory handling, high fecundity and ubiquitous occurrence (Environment Canada, 2000).

The choice of this specie for toxicity evaluation is due to their relevance in aquatic food chains, once it feeds from producers, and it is a main source of subsistence for fish species (Environment Canada, 1990a).

In order to access the toxic effects on *D. magna,* acute and chronic toxicity tests were performed based upon the ISO/DIS 6431 (1996) (Daphtoxkit F^{TM}); and the OECD (1998a) guidelines respectively, being presented in Annexes H and I the respectively procedures.

a) Daphtoxkit F^{TM} magna

This microbioassay is based upon the immobilization rate measurement of the young *Daphnia magna* when placed in toxic solutions. From the dose-response relation, it is possible to calculate the effective sample concentration capable of immobilize 50% of the tested organisms (EC_{50}) (Environment Canada, 2000). As immobilization is the sole biological effect observed, as a consequence, the test's endpoint is the percentage of immobilization at 48 hours. In order to assess the immobilization/mortality % at 48 hours a single concentration, specifically 100% was used.

b) *Daphnia magna* reproduction test

Respectively to the chronic test, the aim of this test is to study the effect of pesticides on the reproductive output of *Daphnia magna* when compared to the controls, *i.e.,* the determination of the lowest-observed-effect concentration and hence the no-observe-effect concentration (NOEC) (OECD, 1998). Consequently the samples D20-1 ("Alverca do Campo" and AlmondaR-2 (Almonda river downstream) were subjected to a dilution gradient of 6.25, 12.5, 25, 50 and 100%.

4.1.6.4 Toxicity test using luminescent bacteria *Vibrio fisherii*

V. fischerii is a bacterium which normally lives in the oceans, and produces blue green light by enzymatic reactions, on a continual if sufficient oxygen is available (Environment Canada, 1992).

Since its development in 1978, the luminescent bacteria toxicity test, distributed commercially as "Microtox", has been used increasingly to assess the toxicity of environmental samples like water or sediments, industrial waste samples; assessment of toxic substances in the environment (Environment Canada, 1992; Ruiz *et al.,* 1997). It was chosen due to its undeniable contribution as a powerful tool, vital in the evaluation of toxicity in environmental samples to assess the impact of pesticides used in agricultural production (Environment Canada, 1992; Ruiz *et al.,* 1997).

This test is particularly useful for exploration or monitoring because it is rapid, simple, uses small samples, rapid response to toxicants, modest laboratory equipment and its inexpensive once the photometer had been already purchased (Environment Canada, 1992; Ruiz *et al.,* 1997).

This bioassay is based on the monitoring of changes in natural light emission (indication of metabolic inhibition in the organisms) of the luminescent *V. fischeri* in contact with toxic compound, under specific condition, measured with a standard photodetection device. Undoubtedly, any toxic action of substances in the sample is presumed to affect metabolic processes of the bacteria, and bioluminescence is inhibited in proportion to the metabolic effect, corresponding to the test endpoint (Environment Canada, 1992, Boluda *et al.* 2002).

However, the availability of organic chemicals and also their potential toxicity to the bacteria can be affected by the interactions between pesticides and different components of the sample (Ruiz *et al*., 1997).

The toxicity endpoint is determined as the effective 50% reduction in the bioluminescence which corresponds to EC_{50} value (expressed in µg/mL) (EPA, 2002; Gosch *et al.* 1997; Boluda *et al.* 2002; Environment Canada, 1997).

The *V. fischeri* Lehmann & Neumann test was ruled according to the Microtox basic test protocol (www.azurenv.com/mtox.htm) .The Microtox toxicity analyzer model 500 (Azur Environmental, Carlsbad, CA, USA) was used to measure the light emission of the luminescent marine bacteria *V. fischeri*.

The bacterium was cultured as a genetically uniform strain and freeze-dried under vacuum (lyophilized).

The bacteria was brought back to an active, living state (reconstituted reagent) by adding reconstitution Reagent and bringing them to a temperature of 5ºC. Subsamples of reconstituted reagent were exposed to concentrations of the sample.

In accordance with the above-mentioned both AlmondaR-2 and D_{20}-1 samples (August 13th) were subjected to a concentration series of 0, 10.24, 20.48, 40.95, 81.90 and 100%, and all samples were adjusted to 2 ± 0.2% NaCl. Tests were run at 15°C and all bioassays were performed according to the validity criteria.

Light measurements were taken after 5, 15 and 30 minute exposures time.

4.1.6.5 Test for survival and growth in sediment using the larvae of freshwater midges - *Chironomus riparius*

In the aquatic environment the sediments provide habitat for many organisms, such as the larval stages and also represent the major repository for the most persistent chemicals introduced in surface waters. Although sediments can be contaminated with high concentration of toxic concern chemicals, there are still a diverse community of benthic or epibenthic organisms that persist without menace. However, it is also possible that sediment may be contaminate with high levels of certain substances and that is not evident at this

point in communities of benthic or epibenthic organisms and no harm to exposed aquatic life can be demonstrated.

Benthic macroinvertebrates, live or depend on the sediment, and many are detritivorous, being responsible for the recycling of organic matter but also for the transfer of contaminants to the water column. Primary consumers (like cladocerans) are filter-feeding organisms and can be useful indicators of the bioavailability of particle-bound contaminants (Environmental Canada, 1997).

The 7 days *C.riparius* Meigen growth test was carried out following the OECD (2004) and ASTM (2002b) guidelines.

Experimental procedures used to prepare spiked sediments are new, varied and not standardized. Ecotoxicological sediments testing began in the late 1970s and there were no standard methods for conducting sediments toxicity tests until the early 1990s.

No single type of bioassay or test organism is suitable for all situations, but the optimal assays vary according to the study and its objectives (Burton, 1991; Ristola, 2000).

This chronic test is designed to assess the effects of prolonged exposure of chemicals to *C. riparius (*Diptera: Chironomidae*),* in order to establish the effect the test substance on the development rate and the total number of fully emerged midges and weight of the larvae.

As a result of the widespread distribution, and common occurrence of this species association with freshwater sediments, together with their ecological significance, easy to culture and test, fast growth, short life cycle, sensitivity to contaminated sediments led to the selection of *C. riparius* for this test.

Other aspect, is related to the midge larvae robust and adaptation to a wide range of conditions (Environment Canada 1997).

The test for survival and growth in sediment using the larvae of freshwater midges *C. riparius,* includes two options to measure the sediment toxicity:

(a)- A static toxicity test, in which the overlying water is not renewed except the loss for evaporation and with continuous aeration – method used for this test;

(b)- A non-static toxicity test, which an intermittent renewal of the overlying water and generally no aeration.

These insects have four life stages: egg, larvae, pupae and adult. The larvae live in the sediment in tubes constructed of algae, sediment particles or other available particles (Environment Canada, 1997; Ristola, 2000).

The test for survival and growth in sediment using the larvae of freshwater Midges - *Chironomus riparius* was based upon the OECD (2004) and ASTM (2002b) guidelines, described in the Annex J.

4.2. Results and discussion

4.2.1 Predicted environmental distribution (PED) through Mackay fugacity model - Level I calculation

For the crops considered in this study and the pesticides registered for these same crops, physical-chemical properties were collected in order to determine the ones that have higher affinity to water based on the Mackay´s fugacity model and affect the aquatic compartment in order to better understand the pesticides impact on surface waters bodies.

It was possible to determine the Predicted Environmental Distribution (PED) for the different compartments, for a total of 123 pesticides of the 146 pesticides at study. Notice that 34% of pesticides present very high affinity to water, i.e., $PED_{water} \geq 80\%$; and 19% of pesticides has high and medium affinity to water, which means that $40\% \leq PED_{water} \leq 80\%$. Furthermore, herbicides are the pesticides group with more affinity to water compartment, when compared to insecticides and fungicides groups (Table 4.17).

Table 4.17 - *Pesticides with medium, high and very high affinity to water compartment.*

	AFFINITY TO WATER				
	Very High (≥80%)		High (≥ 60%-80%)	Medium (≥ 40%-60%)	
Insecticides	acetamiprid	methidation	malathion	methiocarb	
	beta-cyflutrin	methomyl		phosmet	
	carbaryl	oxamyl		thiacloprid	
	carbofuran	pirimicarb			
	cyromazine	pymetrozine			
	dimethoate	thiamethoxam			
	imidacloprid	trichlorfon			
Fungicides	bitertanol	metalaxyl-M	captan	azoxystrobin	myclobutanil
	carbendazim	propamocarb hydrochloride	dimethomorph	chlorothalonil	procymidone
	cymoxanil	propineb	fenamidone	cyazofamid	
	dodine	thiabendazole	pyrimethanil	folpet	
	fosetyl-aluminium	thiram		imazalil	
	mancozeb	ziram		iprovalicarb	
Herbicides	bromoxynil	metribuzin	benoxacor	flufenacet	
	cycloxydim	metsulfuron-methyl	diuron	forchlorfenurom	
	dicamba	nicosulfuron		linuron	
	foramsulfuron	prossulfuron		S-metolachlor	
	glufosinate-ammonium	quinclorac		terbuthylazine	
	glyphosate	rimsulfuron			
	sodium	sulcotrione			
	mesotrione	tribenuron-methyl			

4.2.2 Pesticide risk classification on different ecosystems (hypogean and epygean soil and surface water systems)

The pesticides in study were also classified based on the risk that they represent to different environmental according the different classes: negligible, low, medium, high or very high set in Finizio *et al*. (2000). This rating system allowed the hazard assessment of pesticides at study for the different ecosystems – always based in an integrated approach.

From the total of 146 pesticides at study, it was possible to determine that 108 pesticides present high or very high risk for all the different ecosystems at study. In quantitative terms, insecticides (specifically 55 insecticides) tend to be more dangerous that the other pesticides groups (table 4.18). The tables with the total scores are represented in annex K. With the exception of PRIWS-1, the highest scores were always reached by insecticides. Other important fact is that herbicides were possibly overestimated in PRIES-2 and ERIP indexes due to the high score given by default to phytotoxicity. Fungicides were also overestimated in the ERIP index due to the high score given by default to microorganisms.

Table 4.18 - *Total number of pesticides that presents high or very high risk for the different ecosystems*

INDEXES	INSECTICIDES	FUNGICIDES	HERBICIDES	TOTAL
PRISH-1	11	1	1	13
PRISH-2	6	13	1	20
PRIES-1	4	0	0	4
PRIES-2	0	0	3	3
PRISW-1	14	9	1	24
PRISW-2	16	10	7	33
ERIP	4	2	5	11
Total	55	35	18	108

PRIHS-1: Short-Term Pesticide risk index for the hypogean soil system.
PRIHS-2: Long-Term Pesticide risk index for the hypogean soil system.
PRIES-1: Short-Term Pesticide risk index for the epygean soil system.
PRIES-2: Long-Term Pesticide risk index for the epygean soil system.
PRISW-1: Short-Term Pesticide risk index for the surface water system.
PRISW-2: Long-Term Pesticide risk index for the surface water system.
ERIP: Environmental risk index for pesticides.

In table 4.19 are summarized the pesticides that represent a very high risk for the different ecosystems at study based on the different scores for which one of the system according to the pesticides risk classification, specifically:

- the short-term and long-term risk indexes for the hypogean soil system is classified as “high” or “very high” risk if the PRIHS-1 and PRIHS-2 indexes are above than 40 or 60, and above 30 or 50 respectively;
- the short-term and long-term risk indexes for the epygean soil system is classified as “high” or “very high” risk if the PRIES-1 and PRIES-2 indexes are above than 50 or 70, and above 40 or 70 respectively;

- the short-term and long-term risk indexes for the surface water system is classified as “high” or “very high” if the PRISW-1 AND PRISW-2 indexes are above than 40 or 80, and 30 or 60, respectively;
- the Environmental risk index for pesticides is classified as “high” or “very high” if the ERIP index is above than 40 or 60.

Table 4.19 - *Pesticides that presents very high risk for the different ecosystems at study: hypogean and epygean soil and surface system.*

		Insecticides	Fungicides	Herbicides
Hypogean soil system	Short-term	ethoprophos methidation	-	-
	Long-term	ethoprophos fenazaquine methidation	carbendazime cyprodinil dinocap metiram propamocarb (hydrochloride)	-
Epygean soil system	Short-term and long-term	Without risk		
Surface water system	Short-term	methiocarb	dodine	diuron
	Long-term	bifentrine fenazaquine flufenoxuron pirimicarb	dithianon dodine fenamidone fenbuconazole	metribuzin
Ecosystem		-	-	trifluralin isoxaben

- **Risk classification of pesticides for the hypogean soil system according to PRIHS-1 and PRIHS-2 indexes:**

Only insecticides ethoprophos (score=64) and methidation (score= 64) represents very high risk to hypogean soil system at short-term, respectively for earthworms, beneficial arthropods, and mammals, which had been selected as nontarget organisms representative of the soil system according to the Uniform Principles. The others pesticides expressed in table 4.20 presents high risk (>40 - ≤60) to the hypogean soil system.

Table 4.20 - *Pesticides with high and very high risk for the hypogean soil system (PRIHS-1).*

Insecticides		Fungicides	Herbicides
abamectin	carbofuran	dodine	deltamethrin
spinosad	imidacloprid		
cyfluthrin	methomil		
chlorpyrifos	**ethoprophos**		
dimethoate	**methidation**		
oxydemeton-methyl			

In the figure 4.7 only the pesticides with very high risk for the hypogean soil system at long-term are represented. Fungicides are the group of pesticides that represents both high and very high risk to the hypogean soil system, however the fenazaquin insecticide is the pesticide that embodies the highest risk (score = 68.4) for earthworms, beneficial arthropods, mammals and microorganisms, not considered in the short-term index, selected as nontarget organisms. The insecticides ethoprophos and methidation also represents very high risk to the long-term system.

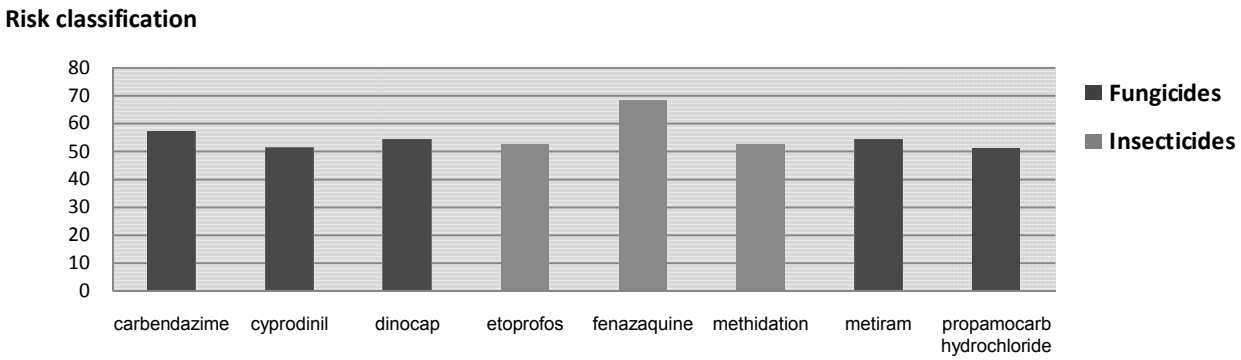

Figure 4.7 - *Pesticides with very high risk (>50) for the hypogean soil system (PRIHS-2).*

- **Risk classification of pesticides for the Epygean soil system according to PRIES-1 and PRIES-2 indexes:**

The PRIES-1 index evaluates the risk for epygean nontarget organisms immediately after a pesticide application, respectively for bees, birds, beneficial arthropods and mammals, selected as non-target organisms representative of the soil system according to the Principle described above.

The insecticides ciflutrine, fenamiphos, imidaclopride and oxamyl represents a high risk to the epygean soil system for a short term. Oxamyl is the insecticide that represents the highest risk for this system (score = 58) (Figure 4.8).

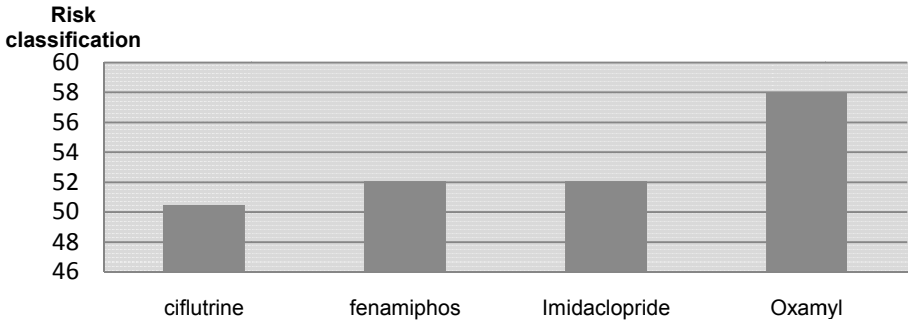

Figure 4.8 - *Insecticides with high risk (>70) for the epygean soil system (PRIES-1).*

The PRIES-2 index (Long-Term Pesticide Risk Index for the Epygean Soil System*)* evaluates the risk for the epygean soil system when a wider time-space scale is considered. Among the relevant organisms, plants, not included in PRIES-1, have been added. It has been assumed that, in the treated area, the crop is not affected (by definition of a plant protection product), while, outside the treated area, an effect on other plant species is likely to occur (Finizio *et al*., 2000).

The herbicides, respectively, isoxaben (score = 46), paraquat dichloride (score = 60) and terbuthylazine (score = 47), represent only a high risk for the epygean soil system (*Figure* 4.9).

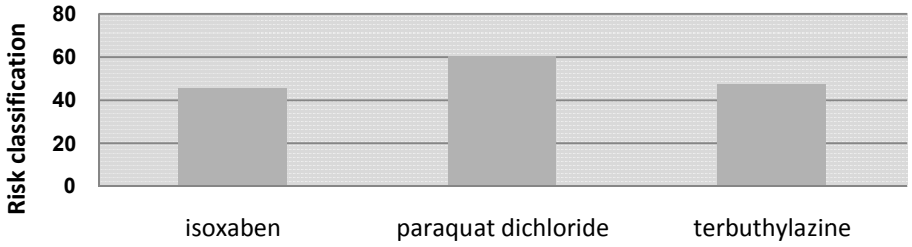

Figure 4.9 - *Pesticides with high risk (>40) for the epygean soil system (PRIES-2).*

Herbicides were also possibly overestimated in PRIES-2 due to the high score given by default to phytotoxicity. It was expected that herbicides would be more likely to reach “high” or “very high” scores for the aquatic environment according Finizio *et al.,* (2000). This is generally due to their lower hydrophobicity and to high algal toxicity.

- **Risk classification of pesticides for the surface water system according to PRIWS-1 and PRIWS-2 indexes:**

The PRIWS-1 index evaluates the immediate risk after pesticides application on a surface water system (1-m depth) adjacent (20 m) to the treated area, for nontarget organisms in surface waters, like algae, Daphnia and fish.

Pesticides in figure 4.10 present high and very high risk for the nontarget organisms in surface waters, being the insecticides the group that represents more risk to this system. Only fungicide dodine (score = 89) and insecticide methiocarb (100) represents a very high risk for this system from the group of pesticides presented in figure 4.10.

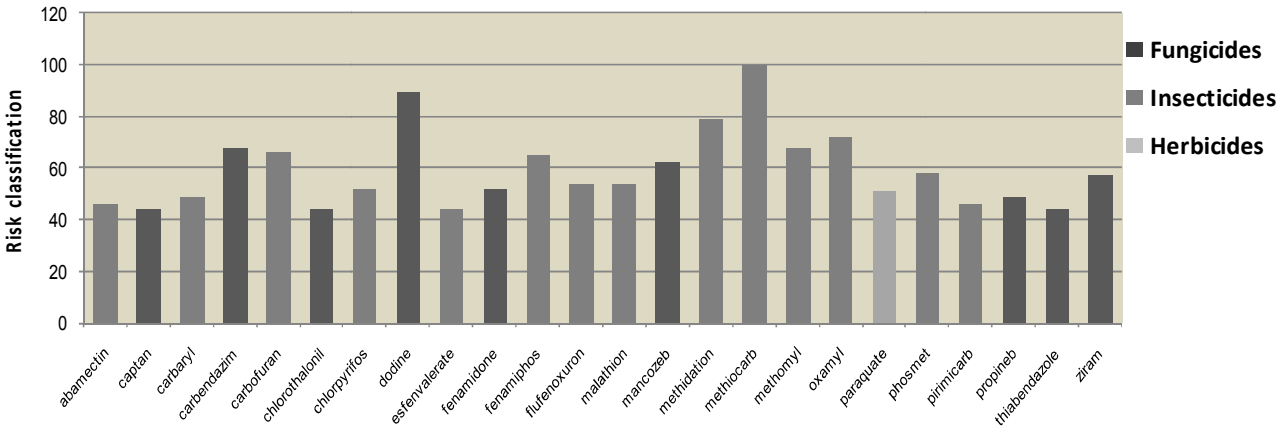

Figure 4.10 - *Pesticides with high (>40) and very high (>80) risk for the surface water system (PRISW-1).*

The PRISW-2 represents the Long-Term Pesticide Risk for the Surface Water System for nontarget organisms in this system.

The pesticides indicated in figure 4.11 represent a very high risk for the nontarget organisms in surface waters. The pesticides which represent the highest risk for this ecosystem are, in crescent order, the herbicide diuron (score=70.4), the fungicide fenamidone (score=70.4) and the insecticide fenazaquin (score=71.5).

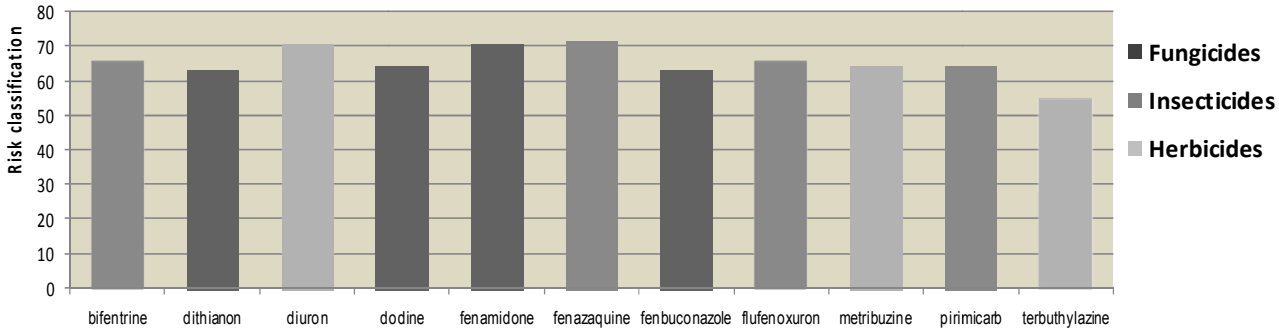

Figure 4.11 - *Pesticides with very high risk (≥ 60) for the surface water system (PRISW-2).*

- **Environmental risk index for pesticides - ERIP**

The Environmental risk index for pesticides is an attempt to give general information about the overall risk for the environment, posed by the use of pesticides.

Pesticides with high and very high risk for the environment represents 13% from total of pesticides, representing herbicides the group with highest risk for the environment.

In figure 4.12 are presented the pesticides with high and very high for the environment, presenting the herbicide trifluralin the highest score (score = 97). The pesticides which could represent high risk for the environment are: the fungicide bupirimate (score = 44), the insecticides chlorpyrifos (score = 44) and tebufenozide (score = 44), and the herbicide oxifluorfen (score = 44). The pesticides that represent a very high risk for the environment are: the insecticides fenazaquin and methoxyfenozide and the herbicides diflufenican, isoxaben, oxiflurfen, trifluralin and terbuthylazine.

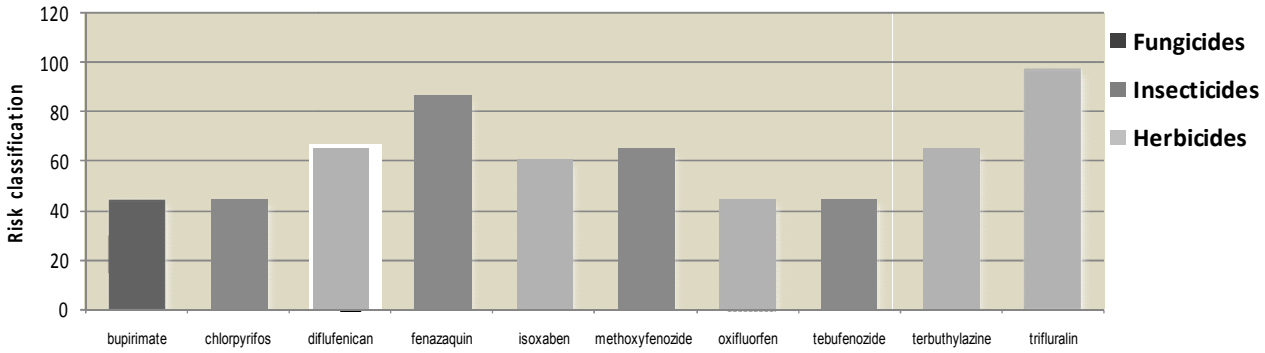

Figure 4.12 - *Pesticides with high (>40) and very high (>60) for the environment (ERIP).*

In table 4.21 are represented the pesticides that could represent high and very risk for more than one environment type. Insecticides are the pesticides group that present a major concern relatively to environment contamination when applied to the crops once its potential to present high and very high is higher than the other pesticides. Considering this pesticides only methidation, carbendazim and dodine present very high affinity to water (>80%) based on the Mackay´s fugacity model which can explain the highest risk of this pesticides to surface water system.

Table 4.21 - *Pesticides with high and very high for the different environments.*

	Insecticides						Fungicides		Herbicide
Indexes	abamectin	cyfluthrin	chlorpyrifos	fenazaquin	methidation	methomyl	carbendazim	dodine	isoxaben
PRISH-1	40	45.5	47.5	-	64	51	medium	45.5	Low
PRISH-2	30.4	40.4	40.4	68.4	52.4	-	57	-	medium
PRIES-1	medium	50.5	medium	-	medium	medium	-	medium	low
PRIES-2	low	-	-	medium	medium	low	low	medium	46
PRISW-1	46	-	52	medium	79	68	68	89	negligible
PRIW-2	medium	-	-	71.5	52	38	56	64	44
ERIP	negligible	-	53	104	negligible	negligible	negligible	negligible	61

- value not calculated

Pyretroid insecticides show in the great part high to very high risk for the aquatic environment in the short term due to their extremely high toxicity for aquatic organisms. The risk is identical in the long term that is the opposite of what was verified in Finizio *et al.* (2000), which can be related in some cases, with the highest affinity for the water compartment and a relative high persistence.

As mentioned above, the main problem encountered in applying this indexes was the difficulty in obtaining reliable data to apply and compare all the indexes, the results in many cases should not be considered real risk classification of pesticides, but an example of application, whose validity is related to the data utilized (Finizio *et al.*, 2000).

General premises can be drawn, such as:

- The higher risk classification for insecticides, in comparison with the other two categories of pesticides, is the result of the structure of the indexes. In general, more weight for the protection of ecosystems was assigned to animals, in comparison with microorganisms and plants.
- The role of persistence is well described by short-term and long-term indexes. For very persistent chemicals (DT_{50}> 3 months), a no negligible risk was always calculated, even for chemicals with very low effects on nontarget organism.

However, several studies show that the synthesis of information on pesticide hazard and exposure into risk indices is found to be useful for providing plausible visions on the status quo of pesticide risks and to identify potential trouble spots where risk reduction might be a main concern. The inclusion in the analysis of a set of indicators representing pesticide hazards along a number of ecological dimensions is also found to be important for articulating trade-offs in management objectives across different environmental concerns. Besides, our empirical analysis confirms that multi-criteria techniques constitute a suitable framework to apply risk indices as decision support tools (Travisi, 2006).

4.2.3 Surface water exposure levels to pesticides

The results of the analysis by SPME and GC-MS enabled the detection of pesticides and metabolites in surface waters of the agricultural area of Almonda subbasin.

For a total of 14 surface waters samples the detection percentage was 100% - at least one or more pesticides and/or metabolites have been detected; 7% out of pesticides and metabolites that were identified have a concentration above 0.1 µg/L and 93% a concentration higher than 0.1 µg/L (parametric value for drinking water – DL nº 243/2001).

Sample sites, number of samples collected, and the frequency of detection for each pesticide in surface water are presented in table 4.22 for the 2008 year.

Table 4.22 - *Pesticides and metabolites monitoring data summary for sample of surface waters (2008)*

Pesticides and metabolites	Number of detections	Frequency of detection (%)	Minimum concentration (µg/L)	Maximum concentration (µg/L)
alachlor	8	57	1.68	10.75
atrazine	6	43	0.28	1.36
metolachlor	6	43	0.21	1.83
propanil	8	57	0.12	7.41
terbuthylazine	14	100	<0.05	3.41
ethofumesate	6	43	<0.05	0.16
chlorpyrifos	7	50	0.06	0.3
3,4-dicloroaniline	8	57	0.84	20.14
E-chlorfenvinphos	6	43	<0.05	<0.05
Z-chlorfenvinphos	8	57	<0.05	0.26

The maximum value detected was 20.19 µg/L for the active substance 3,4-DCA. The herbicide terbuthylazine was the most frequently detected pesticide. It was detected in 100% of the 14 samples taken in 2008. The highest detected concentration of terbuthylazine in surface water was 3.41 µg/L at Almonda River.

Also the pesticides 3,4-DCA, alachlor and propanil were the most frequently detected pesticide. For the three substances the detection percentage was 57%, with maximum detections of 20.19 µg/L, 10.75 µg/L and 7.41 µg/L respectively.

The pesticides with the lowest number of detection were the herbicides atrazine, ethofumesate, metolachlor and the metabolites E-chlorfenvinphos and Z- chlorfenvinphos, with maximum detections of 1.36, 0.16, 1.83, 0.05 and 0.26 µg/L.

In the identified pesticides group mentioned above, the herbicides atrazine and metolachlor are the most commonly detected pesticides in surface water samples from agricultural areas (Rivard, 2003) and in the agricultural area of Almonda subbasin.

Atrazine, metolachlor and alachlor represents the most commonly detected pesticides in surface water samples in the agricultural area of Golegã (Amaral, 2004; Barros, 2005; Bastos 2006). However the metabolite 3,4-DCA was never detected in previous studies in this area.

The dosage levels detected reflect a spatial and temporal of sampling events, probably as the result of common regional mechanisms of pesticide off-site movement to surface water, such as off-site movement in rainfall runoff (Starner, 2003). Another factor that could influence this dosage levels is related with reduced river flow, leading to a more significant pesticides concentration effect, i.e., reduced dilution effect. However, the surface waters contamination by runoff and drainage may contribute for the artificial recharge, resulting from the irrigation and precipitation waters infiltration.

The next figures (4.13, 4.14 and 4.15) represents this variation, however no association was possible between the detected concentration of pesticides and the different periods at study. It is only possible to admit that the dynamic observed could be the result of different application period and the level of the surface waters in the study area, as well edaphical and hydrological features

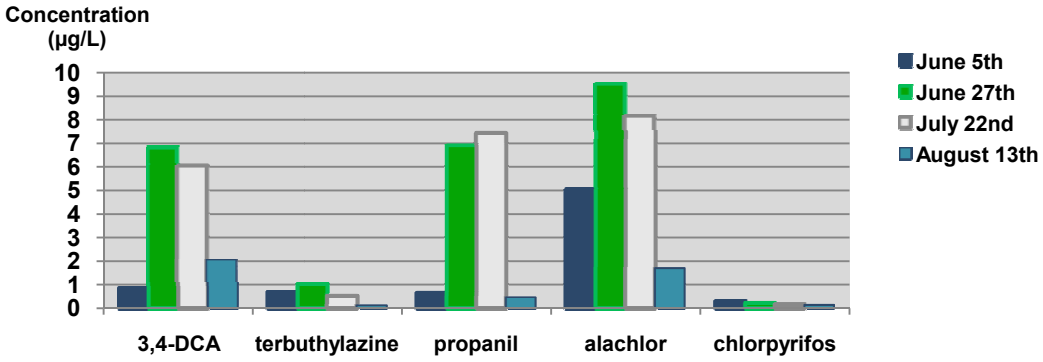

Figure 4.13 - *Evolution of the detected pesticides and metabolites in Almonda River (upstream)- AlmondaR-1.*

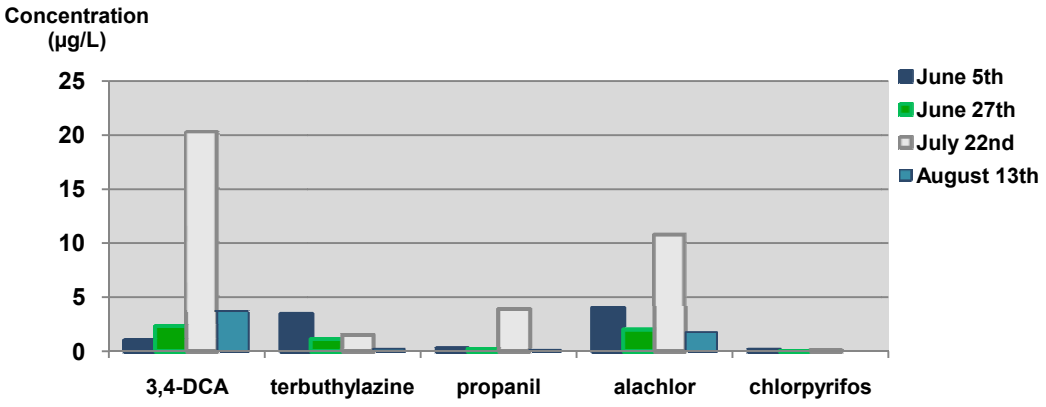

Figure 4.14 - *Evolution of the detected pesticides and metabolites in Almonda River (downstream- AlmondaR-2.*

In Almonda River, as expected, the highest detection concentration of the herbicides alachlor, terbuthylazine and propanil, the insecticide chlorpyrifos and the metabolite 3,4-DCA was observed in upstream of the Almonda river with exception for the herbicide propanil.

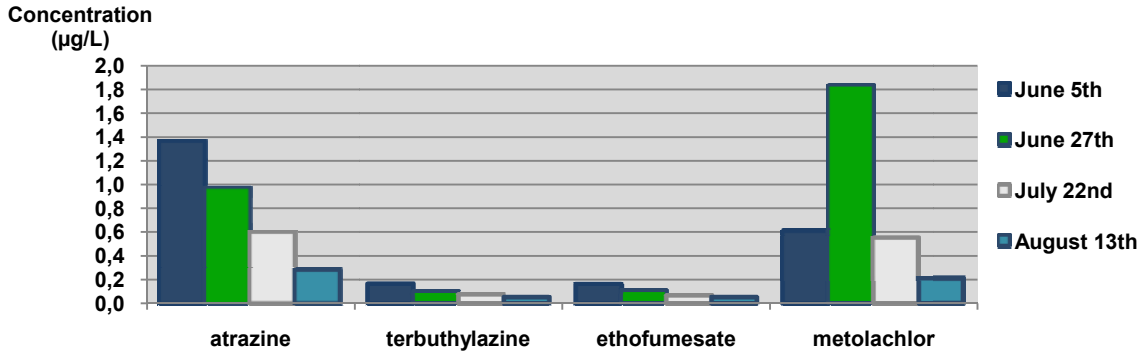

Figure 4.15 - *Evolution of the detected pesticides and metabolites in "Alverca do Campo".*

In "Alverca do Campo" the highest detection concentration of the herbicides atrazine, metolachlor, terbuthylazine and ethofumesate was higher in June 5th, not being detected the same pesticides presented in Almonda river (Figure 4.15).

Considering the pesticides detected, only atrazine has been withdraw from the market in December 2007, the other pesticides are registered in Portugal (DGADR, 2008).

The insecticide chlorpyrifos was detected, however it was not expected once that from the organophosphates insecticides group this is the most unlikely to be transported in runoff, although, in general, OP´s have not been frequently detected in surface waters. Chlorpyrifos, once applied to the soil generally stays in the area where it has been applied, because it sticks strongly to the soil particles. Consequently, it is improbable for the chlorpyrifos to be washed off from the soil and enter local water systems. However, due to the widespread use of the OPs, their use in non-agricultural settings and low detection frequency in most of the reviewed studies, the relation between regional use patterns of Ops and their occurrence in surface waters is unclear (Larson *et al*., 1997).

Atrazine as it is relatively soluble in water (33 mg/L) when applied to the soil surface is subjected to losses by runoff, but without significant meaning (Pereira, 1997).

The herbicide propanil and its metabolite 3,4-DCA were detected above the established by the Water Quality Criteria. 3,4-DCA is exclusively used as an intermediate in the chemical industry for the synthesis of 3,4-dichlorophenylisocyanate and for example the herbicide propanil. Actually, there are no direct uses of 3,4-DCA without chemical transformation. Releases into the environment occur during use of plant protection agents (linuron, diuron, propanil) (European Commission, 2006).

3,4-DCA is normally detected throughout the growing season, being also the parent compound propanil detected, not exceeding generally the reporting level on surface waters. Once propanil is very rapidly degraded in DCA this can explain why 3,4-DCA concentration (20.14 µg/L) is higher than propanil (7.41 µg/L) (Coupe & Thurman, 1997).

As mentioned above also the herbicides alachlor and metolachlor were detected in surface waters. Alachlor belongs to the group of acetanilide herbicides, which metolachlor is part of. This group have moderate to high water solubility and relatively low-sorption coefficients and several are relatively persistent in soil. The detection of alachlor can be the result of its moderate to high potential for loss from fields through surface runoff, primarily in the dissolved phase (Larson *et al., 1997).* Moreover, metolachlor has a very high potential for surface waters contamination since it is relatively mobile and persistent in the soil (Rivard, 2003). For all the samples, alachlor and metolachlor were detected with concentrations above the maximum level admitted for drinking water.

In order to broaden the spectrum of analysis of the sample waters at study, specifically AmondaR-1 and D_{20}-1 sample collected at June 27th were sugbjected a qualitative analysis by GC-MS was performed in "Laboratório de Referência do Ambiente". The following compounds were detected:

AmondaR-1 sample: alachlor, atrazine, carbofuran, dichloropropene, metribuzin, simazine, terbuthylazine and tetrachlroethylene;

D_{20}-1 sample: atrazine, dichloropropene, metolachlor, terbuthylazine, tetrachlroethylene, toluene, trimetilbenzene and xylene.

At this point the next 2 key-points must be emphasized:

- the detected pesticides concentrations, specifically the herbicide alachlor and the insecticide chlorpyrifos exceed the maximum allowed concentration, respectively 0.7 and 0.1 µg/l established by the Water Quality Criteria.

- the pesticides alachlor, atrazine, chlorpyrifos, chlorfenvinphos and simazine are considered priority substances according to the DQA

- metolachlor, atrazine and simazine have been classified as a possible human carcinogen by the U.S. Environmental Protection Agency Office of Pesticide Program's Carcinogenicity Peer Review Committee (Starner, 2003).

Also mixtures of pesticides were observed in all samples, i.e., for each surface water sample at least four substances were detected. In the context of mixtures, it is noteworthy that the 10 pesticides and metabolites detected in surface water in the present study represent very different chemical classes. Thus, when mixtures of pesticides include different chemical classes and, potentially, different modes of action, unexpected toxic effects may

result. This consistent pattern suggests that these chemicals should be evaluated as a single "toxic substance" when assessed from the perspective of environmental effects.

4.2.4 Toxic effects on aquatic organisms in surface waters

In this study, a battery of tests with organisms bearing different key functions at the ecosystem level was used to evaluate the effectiveness surface water toxicity.

The test battery was composed of tests with the bacteria *Vibrio fisherii*, the microalgae *Pseudokirchneriella subcapitata* (Algaltoxkit F^{TM} and microplate technique) and the crustacean *Daphnia magna* (acute and chronic toxicity) upon their respective guidelines as mentioned above.

In the next points will be discussed the toxic effects on *Pseudokirchneriella subcapitata* ("Algatoxkit F") and *Daphnia magna (*acute toxicity) in surface waters:

The results of the microbioassay "Algatoxkit F" are presented in terms of growth inhibition percentage registered during the 72 hours of testing.

For a total of 14 samples 64% shows toxicity to the *P. subcapitata* which corresponds to a growth inhibition % over 50%, being the highest growth inhibition % (100%) observed in Almonda river.

These results suggest that the water in the area of study may have toxic substances for the algal, being the major concern related to the possibility of bioconcentration of these pollutants up the food chain once the *P. subcapitata* is a primary producer in the ecosystems. Furthermore, having a large surface area and hydrophobic cell surfaces can immediately attract and sorbs hydrophobic pollutants. Organisms that feed on algae, like *Daphnia magna*, automatically concentrate more pesticides since they eat large quantities of algae over a longer lifespan (Dunnivant & Anders, 2006).

Comparing Almonda river upstream and downstream results presented in figure 4.16, it is possible to conclude that the effect on the growth of the algae is similar, although the highest % values (100%) were observed in different dates. Yet it is not possible to establish a relationship between the differences in the inhibition growth and the sampling period, as well as the pesticides detected in Almonda river.

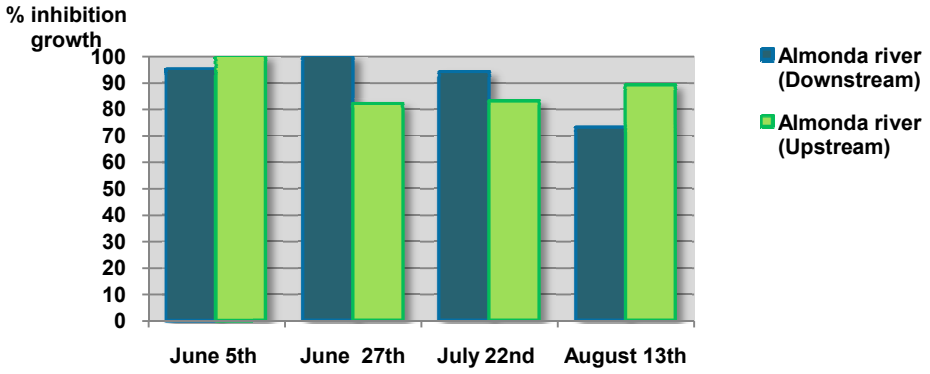

Figure 4.16 - *Growth inhibition % on P.subcapitata for Almonda river.*

Relatively to Alverca do Campo, 17% of the total samples shows toxicity to *P. subcapitata* being the highest growth inhibition observed at August 13th. In the other periods no effects in the algae was observed, suggesting that this samples do not reveal toxicity for the *P. subcapitata*, although the pesticides concentration detected at August 13th were lowest than the other 3 periods sampling (Figure 4.17).

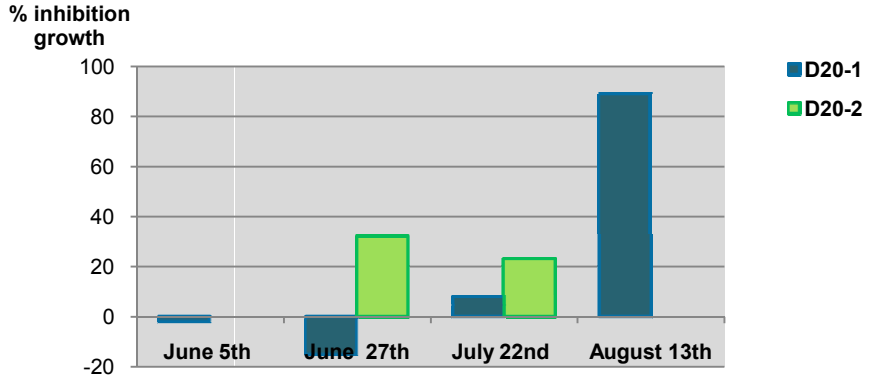

Figure 4.17 - *Growth inhibition % on P.subcapitata for Alverca do Campo.*

In Alverca do Campo, specifically for the D_{20}-1 sample at June 5th and 27th it was observed a negative growth inhibition % which corresponds to a stimulation effect on the *P.subcapitata* growth of 2 and 15%. Although the pesticides concentration presented in Alverca do Campo, specifically atrazine, terbuthylazine, ethofumesate and metolachlor could have some effects on the growth of the algae, these results suggest that the level of toxicity to *P. subcapitata* not only depends on the substances presents in water. This stimulation effect could be related to the possibility that water from Alverca do Campo gather ideal conditions for the growth of the algae like the presence of nutrients, or furthermore the

existence of any other microalgae in samplings able to adapt to the same conditions for the growth of *P. subcapitata*.

Relatively to *Daphnia magna*, the results are expressed as the result of the % of effect on this organism based on the immobilization rate. For a total of 14 samples, 14% shows % of effect to *D. magna* higher than 50%.

In Alverca do Campo the highest effect % was 20% (Figure 4.18), suggesting that this water is not toxic for this aquatic organism once any effect in the mobilization was observed.

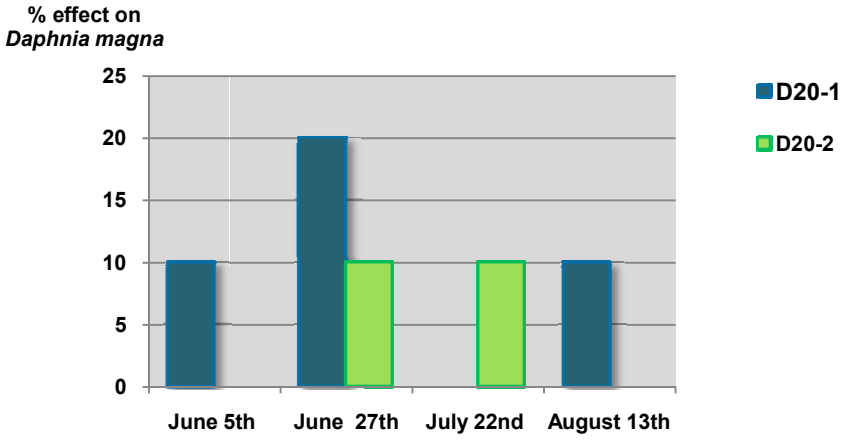

Figure 4.18- *Effect % on the Daphnia magna for Alverca do Campo.*

In Almonda river it was observed the highest % effect on the Daphnia magna, specifically 80% effect on the imobilization rate. The effect % was higher at downstream of Almonda river than upstream, although the pesticides concentration is highest in upstream - AlmondaR-1 (Figure 4.19).

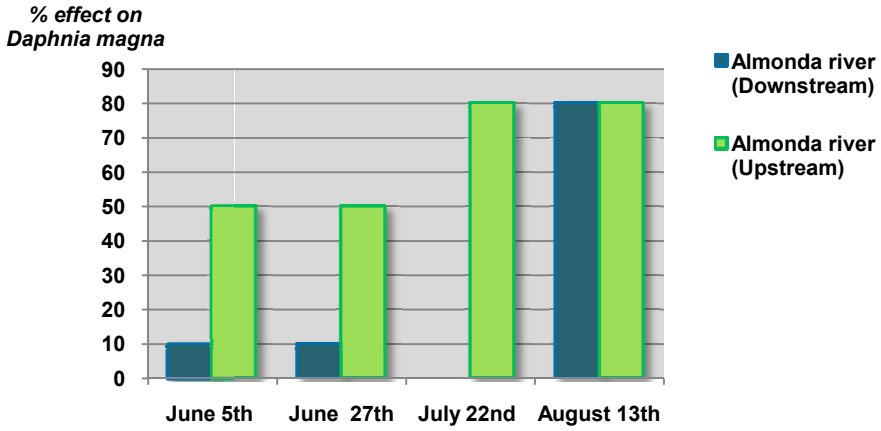

Figure 4.19 - *Effect % on the Daphnia magna for Almonda river.*

At downstream only at August 13th was observed a high % effect on *D. magna* (80%), in the other periods the effect was below than 10%, suggesting that only at August 13th water was toxic to this organism.

At upstream for all the periods was observed % effect ≥ 50%, being the highest % effect at July 22nd and August 13th (80%), suggesting that Almonda river is toxic to *D. magna.*

Using a multi-concentration test to determine the 48-h EC_{50} for the Almonda river (downstream) sample of August 13th, the highest % of effect was obtained from the 100% dilution and also a % of effect of 50% for a 25% dilution (v/v) (*Figure* 4.20).

Resorting to Pearson correlation for the EC_{50} determination, with a level of confidence of 95%, the EC_{50} obtained was 86.06% (v/v).

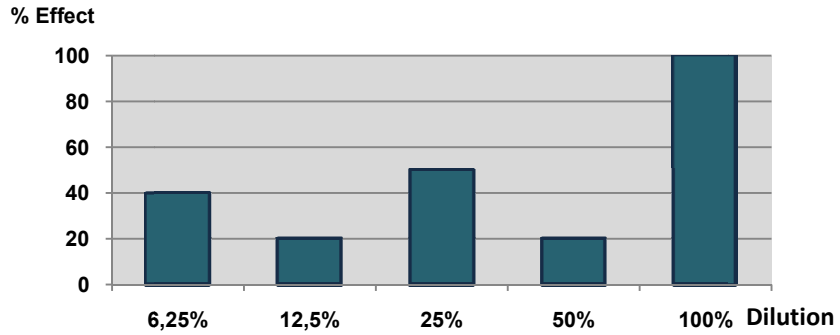

Figure 4.20 - *Effect % on the Daphnia magna for Almonda river (downstream) at August 13th.*

As mentioned above is difficult to establish a relationship between the toxic effects on *P. subcapitata* and *D. magna* based on the pesticides concentration/level exposures in surface waters and the different periods at study (June 5th and 27th, July 22nd and August 13th), resulting in unexpected toxic effects. So, from the perspective of environmental effects is suggested to evaluate the pesticides detected as a single "toxic substance".

In order to accomplish the aquatic toxicity results mentioned above, it was also performed another test battery, specifically with the bacteria *Vibrio fisherii*, the *P. subcapitata* (microplate technique) and the *D. magna* (chronic toxicity). The aquatic toxicity was performed with samples at August 13th of Alverca do Campo and Almonda river (downstream) – AlmondaR-2.

For these tests the data analysis was based on the program Statistica 7 which provided two analyses: an analysis of variance (Anova); and a multiple comparison of treatment means with the control mean (Dunnett´s Procedure) (Zar, 1996).

For all tests, the measured organism responses in the different matrixes (water or sediment dilutions) were examined for significant differences using one-way analysis of variance (ANOVA) or nested ANOVA.

When significant differences were found, the Dunnett's test was performed to test significant differences between the control and the tested dilutions, to determine the no-observed-effect dilution (NOEC) and the lowest-observed-effect dilution (LOEC). Although the Microtox software (Microtox Omni Software 1.18; Azur Environmental) did not provide replicate data to perform an ANOVA, a dilution causing a luminescence inhibition lower or higher than 10% was considered as the NOEC and LOEC value, respectively. The median effective dilutions (EC_{50}) and respective 95% confidence limits (CL) were calculated either through the recommended software (Microtox) or by fitting organism responses to a logistic model using the least squares method (OECD, 1998).

The *V. fischerii* test didn´t revealed toxicity for both samples after 15 min of exposure. For the both samples the effect percentage at 100% of the samples was lower than 10%, consequently NOEC values were considered to be 100% for both samples.

According to Ruiz *et al.* (1997), the bacterium is affected by different chemicals in different ways. Sometimes the toxicity was greater when the time of exposure was increased; this purpose was considered and a preliminary assay was realized for a 30 m exposure time. Consequently, two scenarios were considered: no toxicity is present; or the toxicity was not detected - this last aspect is related with the relatively low sensitivity of the test, which could limit its utility to evaluate the toxicity of water (Pintar *et al.*, 2003). Others limitations are related with the interaction between pesticides and others compounds presents in water (including metabolites and other pesticides) (Ruiz *et al*., 1997).

For *P. subcapitata* significant differences in growth between the control and the tested dilutions, were observed both for samples AlmondaR-2 (one-way ANOVA: $F_{5, 15} = 20$, $P < 0.001$) and Alverca do Campo (D_{20}-1) (one-way ANOVA: $F_{5, 15} = 684$, $P < 0.001$). Although for AlmondaR-2 sample a significant inhibition in growth relatively to the control was observed at all tested dilutions, the NOEC and LOEC values were considered to be 50 and 100% (v/v) of the sample, respectively, because at these dilutions growth was inhibited by just 10% and by 53% respectively; the EC_{50} was 99% (v/v). "Alverca do Campo" sample was shown to be more toxic with NOEC, LOEC and EC_{50} values of 6.25, 12.5 and 50 % (v/v), respectively (Figure 4.21).

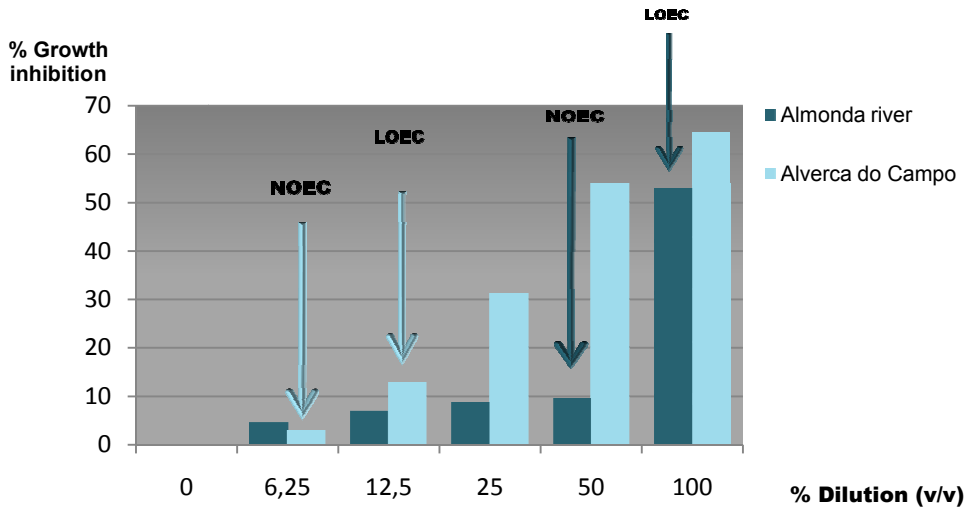

Figure 4.21 - *Growth inhibition % on P.subcapitata for Almonda river (downstream) and Alverca do Campo at August 13th.*

The results from the algal growth tests showed that *P. subcapitata* revealed slight toxicity (72-h) to the both samples, with an EC_{50}= 50% (v/v) to D_{20}-1 sample and EC_{50}= 99% (v/v) to AlmondaR-2 sample. Therefore, a LOEC value was found for sample AlmondaR-2 (50% inhibition at the 100% dilution (v/v)) considering that only the 100% dilution had significant differences in statistical and ecological terms. The summary results are represented in the table 4.23.

Table 4.23 - *Summary of the growth inhibition on the P. subcapitata for Almonda river and "Alverca do Campo".*

SAMPLES	NOEC %(v/v)	LOEC % (v/v)	EC_{50} (LC at 95%) %
AlmondaR-2	50	100	99 (94 -104)
D_{20}-1	6.25	12.5	50 (44-56)

NOEC – no-observed-effect-level; LOEC - lowest-observed-effect-level;
EC_{50} – median effective concentration; LC – level of confidence.

When *D. magna* was exposed to the water samples, significant differences in reproduction, between the control and the tested dilutions were found both for sample AlmondaR-2 (one-way ANOVA: $F_{2,26}$= 11, $P < 0.001$) and sample D_{20}-1 (one-way ANOVA: $F_{4,26}$= 14, $P < 0.001$). Although 100% mortality was observed at 50 and 100% of AlmondaR-2, reproduction was inhibited merely 5% at the 25% dilution (v/v) (though it is expected to be between 25 and 50% dilution). Thus, the NOEC value was 25% (v/v) and it was not possible to estimate the EC_{50}. On the contrary, sample D_{20}-1 showed values of NOEC, LOEC and

EC_{50} of < 12.5, 12.5 and >100% (v/v) respectively. For this sample, percentages of mortality were of 30, 60, 40 and 50% at 12.5, 25, 50 and 100% (v/v) dilutions, respectively (Figure 4.22).

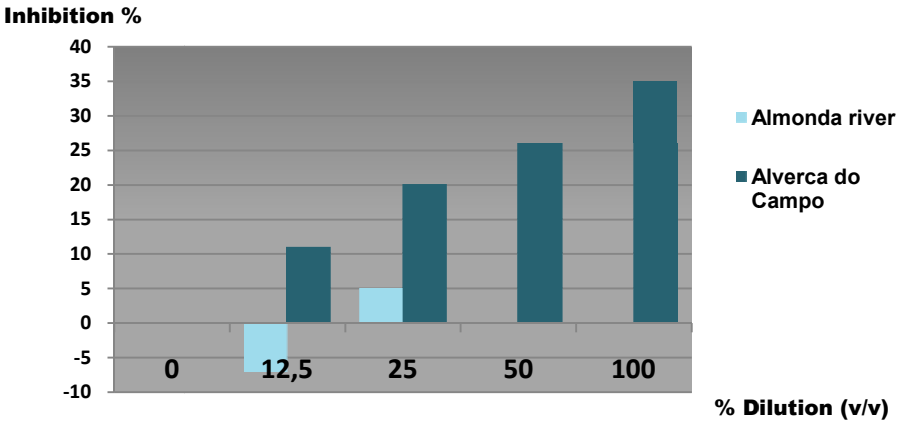

Figure 4.22 - *Inhibition % on D. magna for Almonda river (downstream) and Alverca do Campo at August 13th.*

In the 21-days *D. magna* reproduction test with sample D_{20}-1, a decrease in the number of neonates released was observed at the lowest dilutions.

For the sample AlmondaR-1 the same expected behaviour it was not observed; having an increase in the number of neonates in the 12.5% (v/v) dilution relative to the control. This increase is generally associated with the hormesis effect - low doses of a substance seem to stimulate an apparently beneficial response in the test organism even though larger concentrations lead to a toxic effect. The phenomenon of stimulation of organism response is not uncommon in aquatic toxicology experiments (Bayler & Horis, 1998; Calabrese, 2008).

The results from the *Daphnia* reproductive test showed that Daphnia magna EC_{50}> 100% to D_{20}-1 sample and 25<EC_{50}<50% to AlmondaR-2 sample.

The % of mortality for AlmondaR-2 sample was 100% for the 50 and 100% (v/v) dilutions, enabling the determination of EC_{50}. The % of mortality in the subsequent dilution 25, 13, was respectively 10 and 0%. This can evidence the proximity of the lethal and sublethal toxicity as the result of the range factor (2) for this test. It is suggested for future tests, with AlmondaR-2 sample, the use of lowest range dilutions between the 25 and 50% (v/v), in order to accurate the EC_{50} value. For the 25% (v/v) dilution it is evidenced a significant difference to the control, although the % of inhibition was 5%, enabling the estimation of LOEC.

Considering the NOEC´s and LOEC´s values, the effects on reproduction are less severe for the D_{20}-1 sample. These results suggest that the AlmondaR-2 sample is more toxic when compared to the D_{20}-1 sample (Table 4.24).

Table 4.24 - *Summary of the chronic toxicity results on D.magna for Almonda river and "Alverca do Campo".*

SAMPLES	NOEC (v/v)	LOEC (v/v)	EC50 (LC at 95%)
AlmondaR-2	25%	>25% and < 50%	> 25% and < 50%
D_{20}-1	< 12.5%	12.5%	>100%

NOEC – no-observed-effect-level; LOEC - lowest-observed-effect-level; EC_{50} – median effective concentration; LC – level of confidence.

4.2.5 Toxic effects on aquatic organisms in sediments

For this test the same data analysis mentioned above was performed, based on the program Statistica 7.

For *C. riparius* a significant difference in larval growth between the control and 100% of sample D_{20}-1 was found (nested ANOVA: $_{F1,\ 5}$ = 48, P = 0.001). Yet, growth was not inhibited but rather stimulated by 46% relatively to the control and no mortality to 100% (m/m) dilution was observed, not being observed toxic effects in the 7-days *C. riparius* growth for D_{20}-1 sediment sample. Attending the highest values for coefficient of variation (CV), the existence of outliers was considered. Once detected, the statically relevant was tested, concluding that the existence of outliers do not have express in the CV values.

According to these results, two premises were considered:

-The possibility of the increase in food availability resulting from nutrients present in sediment sample that can provide more nutritive value of the food and of its palatability, once when compared with the control, the weight in the 12.5% (m/m) dilution was significantly higher suggesting insufficient available food; consequently it was expected that the midge weight were significantly highest in the others dilutions.

-the toxicity existence once the weight significantly lowers (38%) in the 50 and 100 % (m/m) dilution relatively to the 12.5% (m/m) dilution, considering that the available food was similarly for the three dilutions.

A second test was performed to investigate these possibilities. The results of the second test showed effectively the sediment was not toxic, however the same stimulation effect was verified rejecting the premise that quantity of food provide was limitative.

Alternatively the presence of nutrients in the sediment sample as a stimulation factor can be considered as a consequence of the behaviour of *C. Riparius* as opportunistic organisms, tolerant to contamination and a high adaptive capacity.

4.2.6 Potential hazard assessment of pesticides in surface waters of the study area

As mentioned above the actual criteria on the potential hazard assessment is based on the concept of toxicity/exposure ratio (TER).

So the potential hazard assessment of pesticides in surface waters to the nontarget organisms, specifically to the algae *P. subcapitata* and the crustacean *D. magna* was based on the comparison between the maximum levels of residues (µg/L) detected on surface waters, and the respectives EC_{50} and NOEC values for this aquatic organisms, presented in table 4.25. The values of EC_{50} (mg/L) and NOEC (mg/L) are based on values from specific bibliographical references (see chapter 4.1.3.1.).

Table 4.25 – *Maximum levels of residues detected (µg/L) on surface waters and EC50 and NOEC (mg/L) values for P. subcapitata and D. magna.*

		P. subcapitata		*Daphnia magna*		
	Pesticide	**EC_{50} (mg/L)**	**NOEC (mg/L)**	**EC_{50} (mg/L)**	**NOEC (mg/L)**	**Maximum levels of residues (µg/L)**
"Alverca do Campo "	**atrazine**	**0,059**	**0,1**	**85**	**-**	**1,36**
	etofumesato	**3,9**	**6,7**	**14**	**0,32**	**0,16**
	metolachlor	**57,1**	**-**	**23,5**	**0,707**	**1,83**
	terbutilazine	**0,016**	**-**	**21,2**	**-**	**0,16**
Almonda river	**alachlor**	**0,966**	**0,02**	**10**	**-**	**10,75**
	propanil	**0,05**	**1**	**4,8**	**-**	**7,41**
	terbutilazine	**0,016**		**21,2**	**-**	**3,41**
	chlorpyrifos	**0,48**	**0,043**	**0,0001**	**0,0046**	**0,30**
	3,4-DCA	**-**	**-**	**-**	**-**	**20,19**
	E-chlorfenvinphos	**-**	**-**	**-**	**-**	**< 0,05**
	Z-chlorfenvinphos	**-**	**-**	**-**	**-**	**0,26**

The maximum levels of pesticides detected on surface waters are under the respective values of EC_{50} and NOEL for the aquatic organisms expressed on table 4.25, so the pesticides detected on surface waters do not represent risk for this organisms, with

exception to insecticide chlorpyrifos. Although the results of the studies on toxic effects on these aquatic organisms reveal toxicity, the risk for these organisms is not too critical.

From the pesticides detected in surface waters samples, accordingly Long *et al.* (2005), chlorpyrifos shows in previous studies to presents very high risk to cause negative impact on surface water quality as a product on the runoff potential and the aquatic toxicity. Although chlorpyrifos was not the pesticide with the highest levels in the analysed samples, probably as a result of soil properties, crop production practices, irrigation management, rain events and pesticides application methods and timing, presents a potential hazard to surface waters in Almonda subbasin.

The obtained toxicity results (see chapter 4.2.4) can be related with the combined presence of these and/or other pesticides, or even other chemicals present in the water masses at study.

Toxicity studies involving pesticides mixtures have resulted in a full spectrum of responses in which the complexity of the interactions could be depended on differences in the chemical properties and modes of toxic action of pesticides (Bailey *et al*., 2000).

Daphnids are the most sensitive species in short term tests; however the results expressed in chapter 4.2.4 shows that *P.subcapitata* was more sensitive than to *D. magna*, which can be explained as the result of the mixture of pesticides in surface waters and the fact the pesticides detected are predominantly herbicides.

The toxicity of the herbicides pesticides group depends on the ability of the toxic molecule to diffuse into the cell membrane and produce some harmful effect based on the principle that herbicide soluble is expected to remain in water and would thus be toxic to planktonic organisms and to macrophytes (Brown & Lean, 1994). However, this intrinsic propriety is not itself sufficient to establish a relationship between the toxicity to aquatic and this type of compound.

5. Mitigation strategies to reduce pesticides inputs into surface water

The contamination of water bodies with agricultural pesticides is responsible for a significant risk to aquatic ecosystems and also drinking water resources. An increasing amount of research has focused on alternative methods in order to reduce surface waters exposure to pesticides.

Mitigation of pesticide inputs into water bodies includes both reduction of diffuse-source (runoff and erosion, tile drainage, spray drift) and of point-source inputs (mainly farmyard runoff) (Reichenberger *et al.*, 2007).

The actual challenge for the pesticide use optimization without compromising the quality and efficiency of farming or consumer and environmental protection is based on combining improvements in chemistry, application technologies and the chemical, physical and biological aspects.

The literature reporting on the effectiveness of mitigation measures in reducing pesticides losses and improving water quality demonstrates that results in terms of reduction of contamination are very variable and can even be contrasting, depending on climate patterns and locations (Reichenberger *et al.*, 2007).

Several projects in Europe have dealt or deal with mitigation of pesticides inputs into water bodies. The recently launched EU-wide TOPPS project is aimed at identifying and disseminating advice, training and information at a larger coordinated scale in Europe with the intention of reducing losses of pesticides into surface water as well as groundwater (Reichenberger *et al.*, 2007).

In Portugal there are several entities at different levels to protect the environment and human health from potential contamination with pesticides due to the actual Europe exigencies. The 6th Framework Program of Action on the Environment due the importance of this problem had establish the pesticides sustainable use in order to assure security conditions in the application, distribution and marketing of products to protect the applicator, consumer and the environment.

Also the implementation of the D.L. No 173/2005 of 21 October, which regulates the activities of distribution, sale, represents a vital tool to provide services for the implementation of plants protection products and its application by end users, are preparing special training courses for the application of plant protection products with high risk, that will start in 2009. However, the principal instruments that affect the pesticides use are the Water Framework Directive (2006 / 60/EC) and Uniform Principles (91/414/EEC).

In order to better protect the water resource and its dependent ecosystems, Brock *et al.* (2006) provides a proposal to harmonize the different scientific approaches for

Ecotoxicology effect assessment adopted in guidance documents that support different legislative directives in E.U. (Water Framework Directive (2006 / 60/EC) and Uniform Principles (91/414/EEC)).

Specifically problems, like pesticides concentrations associated with spray-drift, are often high due to the direct input of pesticides and thus pose significant risk for aquatic fauna (Dabrowski *et al.,* 2005).

The surface waters exposure to pesticides can be relatively easily reduced by the adequacy of practices for irrigation and application of pesticides that would allow a reduction of the elements contaminants from agricultural sources, taking into consideration the productive structure socio-economic reality of the agricultural area of Almonda subbasin.

A reduction in pesticide use is possible through guided pest control with a warning system that informs farmers when to use, for example, insecticides (targeted pest control), through biological control or by means of an integrated approach (Hoevoet *et al,* 2007).

Careful pesticide handling and the execution of as many operations as possible, directly on the treated field, are already highly effective strategies in the role of different strategies that can be taken.

Best management practices can diminish diffuse pesticides inputs to a large extent, but more field experiments should be performed, for example with modelling exercises. Also changing to another pesticide with more favourable physical-chemical or ecotoxicological properties can represents a successful reduce pesticides input to surface waters.

Others possible strategies to reduce the input of pesticides from agricultural areas to water are to increase awareness of the farmers with regard to pesticide handling and application, and to encourage them to implement loss-reducing measures. However, the results of this study that proves that pesticides continues to be presented in surface water in agricultural area of Golegã can be the result of some farmers still do not know or understand that there is a problem with surface water due to pesticides.

So, it is important to promote information campaigns directed to farmers, and also temporary economic compensations for farmers for implementing mitigation measures and personal visits at farms by farm adviser visitors.

Information and advisory campaigns and trainings were found to be sucessful and effective in most pilote catchments, but continuous effort is necessary to prevent backsliding (Reichenberger *et al.*, 2007).

Also a collection system for empty containers is an important strategy, although in Portugal this system should be more attractive to farmers, either through an economic return translated by a discount percentage on the pesticides purchase of or an eco-points system that would reduce the distances travelled, could bring some advantages at this level. The

change in packaging whenever it is possible by plastic water soluble also represents a great strategy to accomplish the pesticides input in water reduction.

Measurement of pesticides in European surface waters during apllication periods have shown that the concentration patterns are highly dynamic and influenced to a significant extent by point sorces. The few continuous monitoring studies showed that dramatic variations in concentrations, can be expected, especially in small catchments driven by runoff processes. Correct pesticide application rates, accurate equipment calibration, proper application timing, careful handling of pesticides, minimizing drift, establishing buffer zones around waterways, and proper cleanup and disposal of pesticides minimize the potential for runoff problems associated with pesticide use, accordling Long *et al*. (2005).

Monitoring and modeling the fate of pesticides and their effects will support the development of an environmentally friendly use of pesticides. Improvements in monitoring pesticides in surface waters based on better understanding of the underlying processes, will develop monitoring strategies in the EU and Portugal as part of the implementation of monitoring programmes in the Water Framework Directive.

In table 5.1 are summarized the most effectiveness and praticability of mitigation measures at the farm and catchement scales.

Table 5.1 - *Mitigation measures at the farm and catchement scales (adapted from Reichenberger et al. (2007)).*

Runoff/erosion	Drift	All diffuse sources	Point sources
Application rate reduction	Application rate reduction	Product substitution	Information campaigns
Shifting application to earlier or later date	No-spray buffers		Filling and cleaning operations on a biobed
Buffer strips at lower field edge	Natural windbreaks		Filling and cleaning operations on the field
Riparian buffer strips	Riparian buffer strips Low (no foliage)		Sharing equipment or spraying by contractors
Constructed wetlands	Spray additives and formulations		Regular inspection of sprayers
Grassed waterways	Drift-reducing nozzles		
Conservation tillage Runoff			
Ground cover			

6. Conclusions and future developments

6.1. Conclusions

The main goal of this study was to assess the quality of the surface waters in the Almonda SubBasin based on an ecotoxicological approach, which included for the aquatic compartment the risk characterisation. Considering the protection zones with special statute in this basin, the "Reserva Natural do Paul do Boquilobo" is the most important representing a vast set of significant values of great fauna and flora biologic productivity. Since 1981 and 1986 this natural area was classified as Biosphere Reserve (UNESCO) and Humid Zone with International Importance (RAMSAR Convention).

In this perspective, and considering the environment parameters that the "production quality" includes, it is important to considerer the impact agriculture practices evaluation, namely the pesticides impact usage in the hydrological and sediment resources.

The agriculture area in study in previous studies showed to be a vulnerable area to pesticides contamination. Today, problems linked to pesticides surface waters contamination are still affecting this area. Being under a high-pressure agricultural area, it is important to continue assessing the pesticides impact in terms of exposure and effects studies, supported by the risk management.

Surface waters samplings and also sediment were done between June and August of 2008, in Almonda river and Alverca do Campo.

Using environmental exposure models and risk indexes allowed the pesticides evaluation with higher environmental risk for the pesticides registered for the crops at study: potato, maize and fruit trees in Almonda subbasin.

As a result, for a total of 142 pesticides analysed it was possible to determine the Predicted Environmental Distribution (PED) of 123 pesticides, from which 42% present high affinity to the water compartment (PED ≥ 80%).

Based on the rating system pesticides risk index for the different systems (hypogean, epygean and water) it is possible to affirm that insecticides and fungicides are the pesticides group that represents high risk for the referred systems.

The risk indexes are considered a powerful tool when combined with the previous one once provides information that can represent a powerful tool to the technicians or farmers in the area, assisting them in decision-making, including a pesticides use careful selection taking into accounts the pesticides features and agricultural ecosystems, and consequently reduce the pesticides input to surface waters.

For a total of 14 surface waters samples, the detection percentage was 100% - at least one or more pesticides and/or metabolites in analysis have been detected; 7% out of the pesticides and/or metabolites that were identified have a concentration above 0.1 µg/L and 93% a concentration higher than 0.1 µg/L - parametric value in water for human consumption (D.L. nº 243/2001).

Besides being present in high quantities, the pesticide residues detected on water samples suggests strong environmental persistence from agricultural application on the field, being the highest pesticides concentration, respectively 36.5 µg/L in Almonda river.

The herbicides terbuthylazine, alachlor, propanil, the insecticide chlorpyrifos and the metabolites 3,4-DCA, E-chlorfenvinphos and Z-chlorfenvinphos were detected in Almonda river. In Alverca do Campo were detected the herbicides atrazine, ethofumesate, metolachlor and terbuthylazine.

The herbicide terbuthylazine was the pesticide with the highest frequency of detection (100%), being the maximum concentration detected at Almonda river (3.41µg/L). Also the 3,4-DCA maximum concentration was detected in Almonda river (20.05 µg/L).

The dosage levels detected reflect a spatial and temporal of sampling events probably as the result of common regional mechanisms of pesticide off-site movement to surface water, such as off-site movement in rainfall runoff (Starner, 2003). Another factor that could influence this dosage levels is related with reduced river flow, leading to a more significant pesticides concentration effect, i.e., reduced dilution effect. So in future studies is recommended to have more sampling periods in order to better understand the pesticides dynamic in this area and reduce the pesticides losses and improving water quality.

In the context of mixtures it is noteworthy that the 10 pesticides and metabolites detected in the surface water samples in the present study represent different chemical classes and consequently unexpected toxic effects. In future these pesticides should be evaluated as a single “toxic substance” when assessed from the perspective of environmental effects.

Based on the dose-response, acute and chronic toxicity for aquatic organisms were evaluated. The organisms studied (*P. subcapitata, D.magna, C.riparius and V. fischerii*) represents the most sensitive indicator species and they are important for the maintenance and viability of aquatic ecosystems.

For a total of 14 samples, 64% shows toxicity to the *P. subcapitata* which corresponds to a growth inhibition % over 50%, being the highest inhibition growth % (100%) observed in Almonda river.

These results suggest that the water in the area of study may have toxic substances for the algal, being the major concern related to the possibility of bioconcentration of these pollutants up the food chain once the *P. subcapitata* is a primary producer in the ecosystems.

The results from the algal growth tests showed that the both samples revealed toxicity to this organism, with an EC_{50}= 50% (v/v) for the Alverca do Campo sample and EC_{50}= 99% (v/v) for Almonda River.

Considering the acute toxicity results for the *Daphnia magna,* it was detected for the Almonda river and Alverca do Campo toxic effects higher than 75% and 80%. Only 36% of the total samples showed effects to *D. magna* and it was only observed at Almonda river.

The results from the Daphnia reproductive chronic test showed that *Daphnia magna* revealed toxicity for both samples, with an EC_{50} *>100*% (v/v) for Alverca do Campo sample and 25%<EC_{50}<50% (v/v) for Almonda river sample (samples from 13th August).

Toxicity tests performed with the bacteria *V. fischerii* did not reveal toxicity for both samples, as well as, no toxic effects were observed in the 7-days *C. riparius* growth. However, it is possible that the sediment may be contaminated with high levels of certain substances without being harmful to the exposed aquatic life.

Consequently, it is valid to affirm that the surface waters in this area present toxicity to aquatic organisms, such as the water flea *Daphnia magna* and the algae *P. subcapitata.* However, it is difficult to connect the effects detected on this species and the pesticides detected; on one hand, because only the detected pesticides were considered; on the other hand, in agricultural areas pesticides at their application peak discharged to water bodies from several agricultural areas can transport a cocktail of diverse pesticides, as mentioned above. So it is impossible to establish a relation between the levels of pesticides found in waters and this toxicity to the samples.

Considering the current state of this area, urgent measures to minimize the continuous impact of the strong agricultural activity must be adopted, in order to preserve the surface waters quality.

The potential risks associated with the contamination of surface waters must and can be alleviated by the adoption of Best Management Practices in the immediate. Many of these are common sense approaches that require relatively little time or money, while others may require significant amounts of both. It is also important to increase awareness of the farmers and more important to promote and disseminate the adoption of protective and integrated production systems.

To monitor the ecological quality of surface waters and to perform realistic risk assessments, ecotoxicological testing and risk assessment needs to be further tuned to the specific dynamics of the pesticides in surface water.

The main goal of the mitigations strategies presented in chapter 5 was the reduction of pesticides losses and water quality improved as well prevention of the pesticides contamination on the surface waters bodies of Almonda Subbasin. However the effectiveness of mitigation measures is very variable depending on environmental compartments interaction and climate patterns, therefore, future studies are recommended in order to accomplish the mitigation strategies real consequence in the agricultural area of Almonda Subbasin.

6.2 Future developments

- ✓ A sustainable use of pesticides is becoming more and more prominent and it demands an effort from the investigation field of the action of pesticides in the environment, a continuous control and a multidisciplinary integration of methodologies and measures aiming for the risk management, especially in areas with high exposure levels to pesticides and a strong agricultural pressure;

- ✓ Enlarging the pesticides detection spectrum by solid phase microextraction (SPME) and gas chromatography coupled to mass spectrometry (GC-MS) to the pesticides that present higher environmental hazard;

- ✓ In the context of mixtures, the detected pesticides in this work should be evaluated as a “single toxic substance” in order to assess the pesticides hazard to non-target aquatic organisms;

- ✓ It is elemental the development of decision support systems, still waiting to be implemented in Portugal, in particular in the study area – which represents an area of extreme vulnerability – so that the agricultural activity can contribute to the national economic and social development without neglecting the environmental quality preservation.

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ANNEX A

Table A.1. *Physical-chemical properties values and environmental partition of the study pesticides (Tomlin, 2003; FOOTPRINT PPDB)*

Active substance INSECTICIDES	MM[a] (g/mol)	SW[a] (mg/L)	VP[a] (Pa)	MP[a] (ºC)	log K_{ow} [a]	H[a] (Pa m^3mol^{-1})	DT_{50} soil [a] (days)
abamectin	873.1	0.007	3.7E-06	161.8-169.4	4.4	2.7E+00	28
acetamiprid	222.7	4250	1.0E-06	98.9	0.8	5.3E-08	5.4
acrinathrin	541.4	0.02	4.4E-08	81.5	5.61	4.8E-02	1001
alpha-cypermethrin	416.3	0.00397	2.3E-05	81.5	6.94	6.9E-02	91
azadirachtin	720.7	26	3.6E-09	155-158	1.09*	-	25
beta-cyfluthrin	434.3	0.0019	1.4E-08	81	5.9	3.2E-03	13
bifenthrin	422.9	0.001	2.4E-05	68-70.6	6	1.0E+02	125
buprofezin	305.4	0.386	4.2E-05	104.6-105.6	4.8	3.3E-02	104
carbaryl	201.2	120	4.1E-05	142	1.85	7.4E-05	28
carbofuran	221.3	320	7.2E-05	153.5	1.52	1.2E-04	60
chlorpyrifos	350.6	1.4	2.7E-03	42-43.5	4.7	6.8E-01	56
chlorpyrifos-methyl	322.5	2.6	3.0E-03	45.5-46.5	4.24	3.7E-01	33
clofentezine	303.1	0.0025	1.4E-07	183	4.1	1.6E-02	132
cyfluthrin	434.3	0.002*	9.6E-07	641	61	1.9E-11	30
cypermethrin	416.3	0.004	2.0E-07	72	6.6	2.0E-02	60
cyromazine	166.2	1300	4.5E-07	224.9	-0.06	5.0E+00	9.7
diazinon	304.3	60	1.2E-04	Not applicable	3.3	6.1E-02	18.4
dicofol	370.5	0.8	5.3E-05	79	4.3	1.6E+00	30
diflubenzuron	310.7	0.08	1.2E-07	228	3.89	4.7E-04	7
deltamethrin	505.2	0.0002	1.2E-08	101	4.61	3.1E-02	23
dimethoate	229.3	2330	2.5E-04	50.75	0.70*	1.4E-06	16
esfenvalerate	419.9	0.002	2.0E-07	59.6	6.22	4.2E-02	287
ethoprophos	242.3	700	4.7E-02	-	3.59	1.4E-02	7
fenamiphos	303.4	40	1.2E-04	49.2	3.3	9.1E-05	1.8
fenazaquin	306.4	0.22	3.4E-06	78.75	5.51	4.7E-03	45
fenoxycarb	301.3	7.9	8.7E-07	53.5	4.07	3.3E-05	31
flufenoxuron	488.8	0.00152*	6.5E-12*	170.5	4	7.5E-06	42
imidacloprid	255.7	610	4.0E-10*	144	0.57	1.7E-101	0.16667
indoxacarb	527.8	0.2	2.5E-08*	88.1	4.65	6.0E-05	186
lambda-cyhalothrin	449.9	0.005	2.0E-07	49.2	7	2.0E-02	40
lufenuron	511.2	<0.06*	<4.0E-06	169.05	5.12*	3.4E-02	20
malathion	330.4	1452	5.3E-03	2.85	2.75	1.2E-02	**0.18**
metam-sodium	129.2	722000	Not volatile	Without melting	1	-	**0.009**
methidation	302.3	200*	2.5E-04	39.5	2.2	3.3E-04	18
methiocarb	225.3	27	1.5E-05	119	3.08	1.2E-04	**35**
methomyl	162.2	57900	7.2E-04*	78.5	0.093	2.1E-06	8
methoxyfenozide	368.5	3.3	<1.5E-06	204.5	3.7	1.6E-04	268
oxamyl	219.3	28000*	5.1E-05*	101	-0.44	3.9E-08	7
oxydemeton-methyl	246.3	miscible	3.8E-03	-20	-0.74	1.0E-05	5
phosalone	367.8	3.052	<6.0E-05*	45	4.01	7.4E-03	4
phosmet	317.3	252	6.5E-5*	72.35	2.95	8.3E-04	**7**
pirimicarb	238.3	30002	<4.0E-04*	91.6	1.7	3.6E-05	234
pymetrozine	217.2	290*	4.0E-06*	217	-0.18	**3.0E-06**	69
spinosad	732	**235***	3.0E-08*	84-99.5	**4**	-	0.5
spirodiclofen	411.3	50*	3.0E-07*	94.8	5.8	2.5E-03	**7.3**
tau-fluvalinate	502.9	0.00103	9.0E-11*	Not applicable	4.26*	4.0E-05	92
tebufenozide	352.5	0.83*	<1.6E-07*	191	4.25	6.6E-05	**348**
teflubenzuron	381.1	0.01	1.3E-08	218.8	4.3	**7.0E-03**	84
tefluthrin	418.73	0.016	8.4E-03	44.6	6.47	2.0E-02	**38.4**
thiacloprid	252.7	185	3.0E-10	136	1.26	4.1E-10	7
thiamethoxam	291.7	4100	6.6E-09	139.1	-0.13	4.7E-10	51
trichlorfon	257.4	120000*	2.1E-04	78.5	0.43	4.4E-07	**18**

Active substance FUNGICIDES	MM[a] (g/mol)	SW[a] (mg/L)	VP[a] (Pa)	MP[a] (ºC)	log K_{ow} [a]	H[a] (Pa m^3mol^{-1})	DT_{50} soil [a] (days)
azoxystrobin	403.4	6	1.1E-10	116	2.51	7.3E-09	14
benalaxyl	325.4	28.6	6.6E-04	78-80	3.54	6.5E-03	77
benalaxyl-M	325.4	33	6.0E-05	76	3.67	2.3E-04	124
bitertanol	337.4	2.7	2.2E-10	1.40E+02	4.10E+01	2.0E-08	23
bupirimate	316.4	13.06	1.0E-04	50-51	3.9	1.4E-03	90
captan	300.6	3.3	1.3E-03	178	2.8	2.0E-04	1
carbendazim	191.2	8	1.5E-03	304.5	1.51	3.6E-03	32
chlorothalonil	265.9	0.81	7.6E-05	252.1	2.92	2.5E-02	28
copper (hydroxide)	97.61	5.10E-06	1.0E-09*	2291	0.44*	-	2600
copper oxychloride	213.6	1.191E-05	1E-08*	2401	0.44*	-	10000
copper sulphate	249.7*	10000*	3.4E-13*	1471	0.44*	-	1600
cyazofamid	324.8	0.107	1.3E-05	152.7	3.2	4.0E-03	5
cymoxanil	198.2	780	1.5E-04	160-161	0.59	3.3E-05	9
cyprodinil	225.3	13	5.1E-04	75.9	4	6.6E-03	45
difenoconazole	406.3	15	3.3E-08	82-83	4.4	1.5E-06	150
dimethomorph	387.9	49.2	9.7E-07	137.2	2.63	5.4E-06	53
dinocap	364.4	0.151	3.3E-06	-22.5	4.54	1.3E-03	24
dithianon	296.3	140*	2.7E-09	215.5	3.2	5.7E-06	**35**
dodine	287.4	630	1.0E-05	136	1.65	1.7E-03	22.3
famoxadone	374.4	0.052	6.4E-07	141.8	4.65	4.6E-03	28
fenamidone	311.4	7.8	3.4E-07	136.8	2.8	5.0E-06	97
fenarimol	331.2	13.7	6.5E-05	118	3.69	1.5E-03	130
fenbuconazole	336.8	3.77	3.4E-04	126.75	3.23	3.0E-05	306
fluazinam	**465.1**	0.135	7.5E-03	116	4.1	4.1E-01	26.5
fludioxonil	248.2	1.8	3.9E-07	199.8	4.12	5.4E-05	25
fluquinconazole	376.2	1	6.4E-09	192.45	3.24	2.1E-06	300
flusilazole	315.4	54	3.9E-05*	54	3.74*	2.7E-04*	95
folpet	296.6	0.8	2.1E-05*	178.5	3.11	7.8E-03	4.3
fosetyl-aluminium	354.1	111300	<1.0E-07[1]	215	-2.4	3.2E-51	0.625
imazalil	297.2	22.4	1.6E-04	52.7	3.82	2.6E-04	5
iprovalicarb	320.4	6.8	3.5E-08	183	3.18	1.3E-06	17
mancozeb	271.2	6.2	<1.3E-05*	172	0.26	5.9E-04	1
metalaxyl-M	279.3	26000	3.3E-03	-38.7	1.71	3.5E-05	21
metam-sodium	129.2	722000	Not volatile	-	1	-	0.009
metiram	1088.6	-	< 1.1E-05	156	0.3	5.4E-03	6
myclobutanil	288.8	132	2E-04*	70.9	2.94	4.3E-04	33
penconazole	284.2	732	1.7E-04*	60.65	3.72	6.6E-04	343
procymidone	284.1	4.52	1.1E-02*	166.25	3.14	2.6E-03	84
hydrochloride	224.7	500000	3.8E-05*	64.2	-1.21	8.5E-9*	30
propiconazole	342.2	100*	2.7E-05*	-23	3.72	9.2E-05	70
propineb	289.8	10*	1.6E-10*	150	-0.26	3.4E-08	**3**
pyrimethanil	199.3	121*	2.2E-03*	96.3	2.84	3.6E-03	54
quinoxyfen	308.1	0.047	1.2E-05	106.75	4.66	3.1E-02	454
sulfur	32.1	insoluble	5.3E-04*	114.5	-	-	-
tebuconazole	307.8	36*	1.7E-06*	105	3.7	1.0E-05*	28
tetraconazole	372.1	156*	1.8E-04*	6	3.56	3.6E-04	-
thiabendazole	201.3	30	4.6E-07	297.5	2.39	2.7E-08	**724**
thiram	240.4	18	2.3E-03	155-156	1.73	3.3E-02	210
tolylfluanid	347.3	0.9	2E-05*	93	3.9	7.7E-02	2
trifloxystrobin	408.4	610	3.4E-06	72.9	4.5	2.3E-03	9.5
ziram	305.8	9.94*	<1.0E-06	246	1.23	<1.90	1.75
zoxamide	336.6	0.681*	<1.0E-05*	160.25	3.76	6.0E-03	10

Active substance HERBICIDES	MM[a] (g/mol)	SW[a] (mg/L)	VP[a] (Pa)	MP[a] (ºC)	log Kow[a]	H[a] (Pa $m^3 mol^{-1}$)	DT_{50} soil [a] (days)
amitrole	84.1	26400	3.30E-08	158	**-0.972**	1.8E-08	182
benoxacor	260.1	20*	5.9E-04*	107.6	-0.46	7.7E-03	5
bentazone	240.3	570	5.40E-06	138	-0.46	7.2E-05	12
bromoxynil	276.9	89*	1.1E-05*	194-195	1.04	5.3E-04	1
cycloxydim	325.5	53	1.0E-05	41	1.36	6.1E-05	1
dicamba	221	25000*	1.7E-03	115	-1.88	6.1E-05	14
diflufenican	394.3	0.05	4.3E-06	160	4.9	1.2E-02	282
diquate	**344.05**	**718000***	**1.0E-05**	**325**	**-4.6**	**5.0E-12**	**365**
diuron	233.1	37.4	1.1E-06	37.4	2.85	5.2E-05	**180**
fluazifop-P-butyl	383.4	1.75	4.1E-04	-15	4.95	1.1E-02	28
flufenacet	363.33	56*	**9.0E-05**	**78**	**3.2**	**9.0E-04**	**32**
foramsulfuron	452.4	3300	4.2E-11*	199.5	-0.78	5.8E-121	9.4
forchlorfenuron	247.7	39	4.6E-08*	165.5	3.2	1.3E-82	10
glufosinate-ammonium	198.2	1370000	1.0E-04	215	0.1	4.5E-09	20
glyphosate	186.1	144000	9.0E-06	190	-3.7	1.2E-08	130
glyphosate (isopropilamonium)	228.2	1.05E+06	2.1E-06*	153.5-206	-5.4	4.6E-10*	47
iodosulforon-methyl-sodium	529.2*	25000*	6.7E-09*	152	-0.7	2.3E-111	10
isoxaben	332.4	1.42	5.5E-07*	176-179	3.94	1.3E-04	120
linuron	249.1	63.8*	5.1E-05*	94	3	2.0E-41	67
mesotrione	339.3	2200	5.7E-06	165	-1	5.1E-07	**17**
metribuzin	214.3	1050*	5.8E-05*	126	1.6	1.0E-05	60
metsulfuron-methyl	381.4	2790	3.3E-10	162	0.018	4.5E-11	52
nicosulfuron	410.4	70	8.0E-10	170.5	-1.8	1.5E-11	43
oxifluorfen	361.7	0.116*	2.7E-05*	85.5	4.47	**2.4E-02**	55
paraquat dichloride	257.2	620000*	<1.0E-05*	340	-4.5	4.0E-09	**2800**
pendimethalin	281.3	0.331	1.9E-03*	56	5.2	2.7E+00	120
profoxydim	466	5.31*	1.7E-04*	-	3.9	1.8E-02	13
propaquizafop	443.9	0.00063	4.4E-10*	66.3	4.78	9.2E-08	26
prosulfuron	419.4	29	3.5E-06	155	-0.21	3.0E-04	23
quinclorac	242.1	0.065*	<1.0E-05*	274	-0.74	**3.7E-02**	**540.5**
quizalofop-P-ethyl	372.8	0.61*	1.1E-07*	76.6	4.61	6.7E-05	0.91667
rimsulfuron	431.4	10	1.5E-06	172.5	-1.47	8.3E-08	20
S- metolachlor	283.8	480*	3.7E-03*	-61.1	3.05	**2.2E-03**	30
sulcotrione	328.8	165	5.0E-06	139	<0	1.0E-05	11
terbuthylazine	229.7	8.5*	-	177-179	3.21	4.1E-03	60
tribenuron-methyl	395.4	2040*	5.2E-08*	142	0.78	1.0E-08	5.1
trifluralin	335.3	0.221	6.1E-03*	48.75	4.83	1.5E+01	126

MM -Molar mass; SW - Solubility in water; P - Vapour pressure; MP - Melting point; log KOW - logarithmic of the partition coefficient octanol-water; H - Henry constant; DT50 - half-time in soil

(a) Tomlin (2006), Bold: pesticides from database online "Footprint"

* values measured at temperatures different than 20º C and/or pH different of 7

- No available data

ANNEX B

Table B.1. Values of the toxicological characteristics to the select registered pesticides *(Tomlin, 2003; FOOTPRINT PPDB)*

Active substance	ORAL*	SKIN and EYE*	INHALATION*	NOEL*
INSECTICIDES	LD_{50} (mg/Kg)	LD_{50} (mg/Kg)	LC_{50} (mg/L air)	mg/kg
acetamiprid	**213**	**2000**	**1.15**	**15**
acrinathrin	**5000**	**2000**	**1.6**	**2.4**
alpha-cypermethrin	57	>2000	>0.593	1.5
azadirachtin	>5000	>2000	0.72	**1000**
beta-cyfluthrin	11	>5000	0.1	60
bifenthrin	54.5	>2000	-	1.5
buprofezin	2198	>5000	>4.57	0.9
carbaryl	264	>2000	3.28	200
carbofuran	8	>2000	0.075	10
chlorpyrifos	135	>2000	>0.2	0.1
chlorpyrifos-methyl	1100	>2000	>0.67	0.1
clofentezine	>5200	>2100	>9	40
cyfluthrin	20	>5000	0.5	50
cypermethrin	138	>2460	2.5	0.05
cyromazine	3387	>3100	>2700	300
diazinon	80	540	>2330 mg/m^3	0.015
dicofol	595	>2500	>5	0.22
diflubenzuron	>4640	>2000	>2.88	40
deltamethrin	>300	>2000	2.2	1
dimethoate	160	>2000	>1.6	0.2
esfenvalerate	75	>2000		>2
ethoprophos	55	26	123 mg/m^3	100
fenamiphos				
fenazaquin	134	>5000	1.9	0.5
fenoxycarb	>10000	>2000	>4400mg/m^3	5.5
flufenoxuron	>3000	>2000	>5.1	
imidacloprid	450	>5000		100
indoxacarb	268	>5000	>2	40
lambda-cyhalothrin	56	632	0.06	0.5
lufenuron	>2000	>2000	>2.35	2
malathion	775	>2000	>5.2	500
metam-sodium				
methidation	25	200	140mg/m^3	0.2
methiocarb	25	>2000		60
methomyl	30	>2000	0.258	50
methoxyfenozide	>5000	>5000	>4.3	10
oxamyl	2.5	>2000	0.056	50
oxydemeton-methyl	50	130	427 mg/m^3	0.25
phosalone	120	1500	0.7	2.5
phosmet	113	>5000		40
pirimicarb	100	>500	0.86	3.5
pymetrozine	5820	>2000	>1800 mg/m^3	3.7
spinosad	3783	>2000	>5.18	9
spirodiclofen	2500	2000	5.03	6
tau-fluvalinate	261	>2000	>0.56	1
tebufenozide	>5000	>5000	>4.3	5.5
teflubenzuron	>5000	>2000	>5058 mg/m^3	4.1
tefluthrin	21.8	177	0.037	-
thiacloprid	396	>2000	>2535	1.23
thiamethoxam	1563	>2000	>3720 mg/m^3	
trichlorfon	250	>5000	>2.3	100

Active substance	ORAL*	SKIN and EYE*	INHALATION*	NOEL*
FUNGICIDES	LD_{50} (mg/Kg)	LD_{50} (mg/Kg)	LC_{50} (mg/L air)	mg/kg
azoxystrobin	>5000	>2000	0.69	18
benalaxyl	680	>5000	>4,2	100
benalaxyl-M	2000	2000	4.42	4.4
bitertanol	4300	>5000	>0.55	100
bupirimate	>4000	4800	0.035	15 daily
captan	9000	>4500	0.668	2000 diet
carbendazim	>2500	>2000	6	300 diet
chlorothalonil	**5000**	**2000**	**0.1**	**3**
copper hydroxide	**489**	**2000**	**0.5**	**-**
copper oxychloride	950	>2000	2.83	16
copper sulphate	-	-	-	-
cyazofamid	>5000	>2000	>5,5	17 daily
cymoxanil	760	>2000	>5,06	3,0 daily
cyprodinil	>2000	>2000	>1200 mg/m3	3
difenoconazole	1453	>2010	>3300 mg/m3	1
dimethomorph	3900	>2000	>4.2	9 daily
dinocap	990	>2000	>3	0.4
dithianon	115	>2000	0.33	2.8
dodine	>1000	>1500	1.05	800
famoxadone	>5000	>2000	>5.3	1.2
fenamidone	2028	>2000	2.1	3.6 daily
fenarimol	>200	>2000	2,04 mg tech./air	25
fenbuconazole	2000	>5000	>2,1	6.4
fluazinam	>5000	>2000	0.463	3.48
fludioxonil	>5000	>2000	>2,6mg/m3 air	40
fluquinconazole	112	625	0.754	0.31
flusilazole	674	>2000	3.7	10
folpet	>9000	>4500	1.89	**44.5**
fosetyl-aluminium	>7080	>2000	>5,11	298
imazalil	227	4200	2.43	2.5
iprovalicarb	>5000	>5000	>4977mg/m3(dust)	**196**
mancozeb	>5000	>5000	>5,14	4,8
metalaxyl-M	375	>2000	>2290mg/m3	250
metam-sodium				
metiram	>5000	>2000	>5,7	3.1
myclobutanil	1600	>5000	5.1	-
penconazole	2125	>3000	>4000 mg/m3	0.71
procymidone	6800	>2500	>1500mg/m3	300
propamocarb hydrochloride	1400	>3000	>5.54	26
propiconazole	1490	>4000	>5800 mg/m3	3.6
propineb	>2500	>5000	>0,7 (aerosol)	50
pyrimethanil	4150	>5000	>1,98	20
quinoxyfen	>5000	>2000	>3,38	20
sulfur	>5000			
tebuconazole	1700	>5000	0,37 (aerosol)	20
tetraconazole	1031	>2000	>3,66	80
thiabendazole	3100	>2000	>0,5	10
thiram	210	>2000	4,42(4h)	1,5 (2 y)
tolylfluanid				
trifloxystrobin	>5000	>2000	>4646 mg/m3	
ziram	100	>2000	0.07	T+; R26|Xn;
zoxamide	>5000	>2000	>5,3	50

Active substance	ORAL*	SKIN and EYE*	INHALATION*	NOEL*
HERBICIDES	LD_{50} (mg/Kg)	LD_{50} (mg/Kg)	LC_{50} (mg/L air)	mg/kg
amitrole	5000	2500	0.439	-
benoxacor	>5000	>2010	>2	0.5
bentazone	500	>2500	>5.1	10
bromoxinil	81	>1000	0.15	20ppm (2y)
cycloxydim	5000	>2000	>5.28	7
dicamba	1707	>2000	>9.6	110 (2y. daily)
diflufenican	>2150	>2000	>5.12	1000
diquat	**218**	**424**	**0.125**	**8.9**
diuron	437	5000		0
fluazifop-P-butyl f.h	2451	>2000	>6.06	1
flufenacet	598	2000	3.74	1.67
foramsulfuron	5000	2000	5.04	-
forchlorfenuron				
glufosinate-ammonium	416	2000	1.26	64
glyphosate	3530	>5000	>4.98	400
glyphosate (isopropilamonium)	5700	>5000	>1.3	300
iodosulforon-methyl-sodium	**2678**	**2000**	**2.81**	**7**
isoxabena	>5000	>2000	>1.99	5.6
linuron	1500	>2000	>4.66	2
metam-sodium				
metribuzin	250	>20000	>0.65	100
metsulfuron-methyl	5000	5000	1.3	-
nicosulfuron	**5000**	**2000**	**5.47**	**358**
oxifluorfen	>5000	>10000	>5.4	2
paraquat dichloride	30	200	0.6	1.7
pendimethalin	**3189**	**2000**	**320**	
profoxydim	**5000**	**4000**	**5.2**	**4**
propaquizafop	**5000**	**2000**	**2.5**	**6.25**
prosulfuron	**986**	**2000**	**5.4**	**3**
quinclorac				
quizalofop-P -ethyl	1182	5000	5.8	1.3
rimsulfuron	>5000	>2000	>5.4	3000
s- metolachlor	2000	2000	2.91	15
sulcotrione	>5000	>4000	>1.6	100ppm
terbuthylazine	1590	>2000	>5.3	15.4
tribenuron-methyl	5000	5000	12	6
trifluralin	>5000	>5000	>4.8	813

* values from Tomlin (2006)

bold- values from "Footprint" online database

- no available data

LD_{50}- Median lethal dose; LC_{50}- Median lethal concentration; NOEL- No Observed Effect level

ANNEX C

Table C.1. Ecotoxicological values for the selected registered pesticides *(Tomlin, 2003; FOOTPRINT PPDB)*

	Birds		Fish		*Aquatic invertebrates*		Algae		Honeybees	Earthworms
Active substance INSECTICIDES	Specie	Acute LD50 mg/Kg	Species/ Test duration (h)	Acute LC_{50} (mg/l)	Species/ Test duration (h)	Chronic EC_{50} (mg/l)	Species/ Test duration (h)	Acute EC_{50} (mg/l)	LD_{50} (µg/honeybee)	Acute LC_{50} mg/Kg soil
abamectin	mallard ducks	84.6	trout	0.0032	*Daphnia magna*	0.34	*Pseudokirchneriella subcapitata*	>1000	**0.0022**	28
acetamiprid	mallard ducks	98	carp	>100	*Daphnia magna*	>200	*Scenedesmus subspicatus*	> 93	14.5	**9**
acrinathrin	**mallard ducks**	**>1000**	**trout**	**5.66**	***Daphnia magna***	**0.57**	***green algae***	**0.82**	**175**	**1000**
alpha-cypermethrin	bobwhite quail	>2025	rainbow trout/96	2.8 ug/l	*Daphnia magna/48*	0.0001	*Pseudokirchneriella subcapitata/96*	>100 ug/l	0.059	**100**
azadirachtin										
beta-cyfluthrin	*Colinus virginianus*	2000	salmon	0.000068	***Daphnia magna***	0.00029	***Scenedesmus subspicatus***	**10**	**0.001**	1000
bifenthrin	bobwhite quail	1800	trout	0.00015	*Daphnia magna*	0.00016	***Scenedesmus subspicatus***	**50**	0.1	>1000
buprofezin	bobwhite quail	>2000	carp	0.527	*Daphnia magna*	0.42	*Pseudokirchneriella subcapitata*	>2.1	**163.5**	500
carbarly	pigeans	1000	trout	1.3	*Daphnia magna*	0.006	*Selenastrum capricornutum*	1.1	1	106
carbofuran	bobwhite quail	2.5	sun fish	1.75	*Daphnia magna/48*	0.0386	***Raphidocelis subcapitata***	**6.5**	**0.04**	13
chlorpyrifos	chicken	32	trout	0.0007	*Daphnia magna*	0.0017	*Selenastrum capricornutum*	>0.4	**0.059**	210
chlorpyrifos-methyl	bobwhite quail	932	trout	0.41	*Daphnia magna*	0.016	*Selenastrum capricornutum*	0.57	0.38	182
clofentezine	mallard ducks	>3000	rainbow trout/96	>0.015	*espécie desconhecida*	0.1	***Pseudokirchneriella subcapitata***	**0.32**	>252.6	215
cyfluthrin	bobwhite quail	>2000	rainbow trout/96	0.00047	*Daphnia magna*	0.0016	*Scenedesmus subspicatus*	>10	0.001	>1000
cypermethrin	chicken	>2000	rainbow trout/96	0.69	***Daphnia magna***	**0.0003**	***Selenastrum capricornutum***	**0.1**	0.035	100
cyromazine	mallard ducks	>1000	sun fish/96	>90	***Daphnia magna***	100	*Scedesmus subspicatus*	124	**186**	>1000
deltamethrin	mallard ducks	>4640	rainbow trout/96	0.91	***Daphnia magna***	**0.00056**	*Selenastrum capricornutum/96*	>9,1	0.000079	>1290
diazinon	mallard ducks	2.7	rainbow trout/96	2.6	***Daphnia magna***	**0.001**	*Selenastrum spp*	>1	0.09	65
dicofol	*Coturnix japonica*	1418	minnows/96	0.183	***Daphnia magna***	0.14	*Scedesmus subspicatus/96*	0.075	>50 ug tech/bee	43.1
diflubenzuron	Unknown species	2000	rainbow trout/96	>0.2	*Daphnia magna*	0.0071	*Raphidocelis subcapitata*	0.3	>100	500
dimethoate	pheasants	14.1	sun fish/96	17.6	*Daphnia magna/48*	2	*Selenastrum capricornutum/72*	90.4	0.15	99.5
esfenvalerate	bobwhite quail	381	sun fish/96	0.26 ug/l	Unknown species	0.0009	Unknown species	0.0065	0.06	212.5
ethoprophos	hens	5.6	sun fish/96	2.1	*Daphnia magna*	0.2	Unknown species	28.3	**5.56**	39.6
fenamiphos										
fenazaquin	bobwhite quail	1747	trout/96	3.8ug/l	***Daphnia magna***	0.0041	***Scenedesmus subspicatus***	**49**	**1.21**	26.5
fenoxycarb	japanese quail	>7000	rainbow trout/96	1.6	***Daphnia magna***	0.5	*Scenedesmus subspicatus/96*	1.1	0.1	425
flufenoxuron	bobwhite quail	>2000	rainbow trout/96	>4.9 ug/l	*Daphnia magna/48*	0.00004	*Selenastrum capricornutum/96*	24.6	>109.1	>1000
imidacloprid	japanese quail	31	rainbow trout/96	211	***Daphnia magna***	85.2	*Pseudokirchneriella subcapitata/72*	>100	**0.0037 oral**	10.7
indoxacarb	bobwhite quail	98	rainbow trout/96	0.65	***Daphnia magna***	0.6	***Raphidocelis subcapitata***	**0.11**	23.33	>1250
lambda-cyhalothrin	mallard ducks	>3950	sun fish/96	0.21	*Daphnia magna/48*	0.00036	*Selenastrum capricornutum/96*	1	**0.038**	>1000
lufenuron	mallard ducks	>2000	bluegill sunfish /96	>29	*Daphnia magna/48*	0.0011	*Green algae/72*	10	>197	>1000
malathion	Colinus virginianus	359	bluegill sunfish /96	0.1	*Daphnia magna/48*	0.001	*72*	13	0.16	613
metam-sodium	*Colinus virginianus*	211	*Lepomis macrochirus*	0.175	***Daphnia magna***	0.99	-	-	-	-
methidation	mallard ducks	23.6	bluegill/96	0.002	***Daphnia magna***	**0.0064**	*Scenedesmus subspicatus/72*	22	**0.13**	5.6
methiocarb	mallard ducks	7.1-9.4	rainbow trout/96	0.436	***Daphnia magna***	**0.008**	*Scenedesmus subspicatus*	1.15	**0.23**	>200
methomyl	northern bobwhite quail	24.2	bluegill sunfish /96	0.63	***Daphnia magna***	0.0076	*Selenastrum capricornutum/72*	>100	0.28	21
methoxyfenozide	bobwhite quail	>2250	rainbow trout/96	>4.2	***Daphnia magna***	3.7	*Selenastrum/96 and 120*	>3.4	100	>1213
oxamyl	mallard ducks	3.16	rainbow trout/96	4.2	***Daphnia magna***	**0.319**	*72h*	3.3	0.38	112
oxidemetão-metilo	bobwhite quail	34	sunfish/96	1.9	***Daphnia magna***	**0.11**	*Scenedesmus subspicatus/ErC50*	49	0.31	115
phosalone	mallard ducks	>2150	rainbow trout/96	0.63	*Daphnia magna/48*	0.00074	*Scenedesmus subspicatus*	**1.1**	**4.5**	22.5
phosmet	*Colinus virginianus*	**57**	bluegill sunfish /96	0.07	***Daphnia magna***	**0.002**	**Unknown species**	**0.07**	1	52
pirimicarb	bobwhite quail	20.9	bluegill sunfish /96	55	*Daphnia magna/48*	0.017	*Selenastrum sp./96*	140	4	>60
pymetrozine	mallard ducks	>2000	rainbow trout/96	>100	***Daphnia magna***	**87**	***Raphidocelis subcapitata. 72***	21.6	>117/48h	>250
spinosad	mallard ducks	>2000	japanese carp/96	3.5	*Daphnia magna*	14	***Navicula pelliculosa***	**0.09**	**0.0029**	>1000
spirodiclofen	*Colinus virginianus*	2000	*Oncorhynchus mykiss*	0.035	*Daphnia magna*	0.051	*Pseudokirchneriella subcapitata*	0.06	196	1000
tau-fluvalinate	bobwhite quail	>2510	rainbow trout/96	0.0027	***Daphnia magna***	0.0089	***Raphdocelis subcapitata***	10	**5.83**	500
tebufenozide	bobwhite quail	>2150	sunfish/96	3	***Daphnia magna***	3.8	*Scenedesmus/96*	0.23	234	>1000
teflubenzuron	mallard ducks	>2250	trout/96	4	***Daphnia magna***	0.0028	***Scenedesmus subspicatus***	0.02	not toxic	1000
tefluthrin										
thiacloprid	japanese quail	49	sunfish/96	25.2	*Daphnia magna/48*	85.1	*Scenedesmus subspicatus /72/ErC50*	97	17.32 oral	105
thiamethoxam	mallard ducks	576	*Oncorhynchus mykiss*	100	*Daphnia magna/48*	>100	*Green algae/96*	>100	0.24	>1000
trichlorfon	*Anas platyrhynchos*	36.8	golden orfen/96	*0.52*	***Daphnia magna***	0.00096	*Scenedesmus subspicatus*	>10	**0.4 oral**	-

	Birds		Fish		*Aquatic invertebrates*		Algae		Honeybees	Earthworms
Active substance FUNGICIDES	Specie	Acute mg/Kg	Species/ Test duration (h)	Acute LC_{50} (mg/l)	Species/ Test duration (h)	Chronic (mg/l)	Species/ Test duration (h)	Acute EC_{50} (mg/l)	LD_{50} (µg/honeybee)	Acute LC_{50} mg/Kg soil
azoxystrobin	bobwhite quail	>2000	trout	0.47	*Daphnia magna*	0.08	*Selenastrum capricornutum*	0.12	>25	283
benalaxyl	mallard ducks	>4500	Trout	3.75	*Daphnia magna*	0.59	*Selenastrum capricornutum*	2.4	>100	180
benalaxyl-M	***Colinus virginianus***	**2000**	***Oncorhynchus mykiss***	**4.9**	***Daphnia magna***	**17**	***Scenedesmus subspicatus***	**16.5**	**104**	**472.7**
bitertanol	quail	776	trou/96	2.14	*Daphnia magna*	4.46	*Scenedesmus subspicatus*	6.52	>104.4	>1000
bupirimate	pigeons	>2700	Trout	1.4	*Daphnia magna*	7.3	***Pseudokirchneriella subcapitata***	**1.6**	50	1000
captan	bobwhite quail	2000	Trout	0.034	*Daphnia magna*	7.00		91	**100**	519
carbendazim	bobwhite quail	5826	carp	0.61	*Daphnia magna*	0.13	*Scenedesmus subspicatus/72*	419	>50	6
chlorothalonil	mallard ducks	>4640	Trout	0.047	*Daphnia magna*	0.07	*Selenastrum capricornutum*	0.21	>63	**268.5**
copper (hydroxide)	mallard ducks	167.3	-		*Daphnia magna*	0.29		>187.5	18.1	>481.6
copper oxychloride	bobwhite quail	600	-		*Daphnia magna*	2.3	-		toxic	155
copper sulphate	mallard ducks	600	***Oncorhynchus mykiss***	13.2	*Daphnia magna/14d*	2.3	***Pseudokirchneriella subcapitata***	**12.3**	**23.5/contact**	155
cyazofamid	mallard ducks	>5000	carp/96	0.14	*Daphnia magna*	0.14	*Selenastrum capricornutum/72*	0.025	>151.7	>1000
cymoxanil	bobwhite quail	>2250	sun fish/96	29	***Daphnia magna***	**27**	*selenastrum capricornutum /5*	1.21	> 25 /contact	>2208
cyprodinil	mallard ducks	>500	sun fish/96	2.17	*Daphnia magna/48*	0.033	*selenastrum capricornutum/72*	5.2	>100	192
difenoconazole	mallard ducks	>2150	rainbow trout/96	0.81	***Daphnia magna***	0.77	*Scenedesmus subspicatus/72*	0.032	>187	>610
dimethomorph	mallard ducks	>2000	rainbow trout/96	6.2	*Daphnia magna/48*	>10.6	*scenedesmus subspicatus/96*	29.2	>32.4	31
dinocap	bobwhite quail	>2150	sun fish	5.3	*Daphnia magna*	0.004	*/72*	>105	6.5	120
dithianon	bobwhite quail	290	carp/96	0.1	***Daphnia magna***	0.26	*/96*	12	**25.4**	**578**
dodine	japanese quail	788	**harlequim fish/48**	0.53	*Daphnia magna/48*	0.13	*Selenastrum capricornutum*	0.0051	>0.2	547
famoxadone	bobwhite quail	>2250	rainbow trout/96	0.011	*Daphnia magna/48*	0.012	*Selenastrum capricornutum/72*	0.022	>25	470
fenamidone	bobwhite quail	>2000	rainbow trout/96	0.74	*Daphnia magna/48*	**0.05**	*Scenedesmus subspicatus/72*	3.84	**>159.8**	25
fenarimol	bobwhite quail	>2000	rainbow trout/96	4.1	*Daphnia magna/48*	0.82	*Raphidocellis subcapitata*	1.5	>10	**250**
fenbuconazole	Duck	2110	sunfish/96	0.68	*Dapnhia magna*	2.3	***Scenedesmus subspicatus/72***	**0.13**	5.2	>100
fluazinam	colinus virginianus	1782	Trout/96h	0.036	*Daphnia magna/48*	<0.22	*Selenastrum capricornutum/96*	0.164	100	>1000
fludioxonil	mallard ducks	>2000	rainbow trout/96	0.5	*Daphnia magna/48*	1.1	*Selenastrum capricornutum/120*	0.092	> 329	>1000
fluquinconazole	mallard ducks	>2000	sunfish/96	1.34	***Daphnia magna***	5	*Selenastrum capricornutum*	0.014	**100**	>1000
flusilazole	mallard ducks	>1590	rainbow trout/96	1.2	*Daphnia magna*	3.4	***Selenastrum capricornutum***	6.4	>150	**388**
folpet	mallard ducks	>2000	*Oncorhynchus mykiss*	0.233	*Daphnia magna*	>1.46	***Scenedesmus subspicatus***	>10	461.8/oral	**1000**
fosetyl-aluminium	bobwhite quail	>8000	bluegill sunfish/96	>60	***Daphnia magna***	**100**	*Scenedesmus pannonicus/90*	21.9	>236 /oral	>1000
imazalil	mallard ducks	510	rainbow trout/96	1.5	***Daphnia magna***	3.5	Algae	0.87	40	541
iprovalicarb	bobwhite quail	>2000	sunfish/96	>20.7	*Daphnia magna/48*	19.8	*Selenastrum capricornutum72*	>10	>199	>1000
mancozeb	*Passer domesticus*	>1290	rainbow trout/96	1	*Daphnia magna/48*	3.8	*Selenastrum capricornutum/120*	0.044	>209	>1000
metalaxyl-M	bobwhite quail	981	rainbow trout/96	>100	***Daphnia magna***	100	*Scenedesmus subspicatus/72*	103	**127**	830
metiram	bobwhite quail	>2150	rainbow trout/96	0.33	*Daphnia magna/48*	0.11	*Chlorella/96*	0.3	>80	>1000
myclobutanil	bobwhite quail	510	rainbow trout/96	2	***Daphnia magna***	17	*Scenedesmus subspicatus/96*	0.91	>171	99
penconazole	mallard ducks	>1590	rainbow trout/96	1.7-4.3	***Daphnia magna***	**6.75**	*Selenastrum capricornutum/5*	0.83	>5	>1000
procimidone	*Colinus virginianus*	4092	rainbow trout/96	7.2	***Daphnia magna***	1.8	***Scenedesmus acutus***	2.6	**100 /contact**	**1000**
propamocarb hydrochloride	mallard ducks	>1842	bluegill sunfish/96	>92	*Daphnia magna*	100	*Selenastrum capricornutum/72*	>85	-	
propiconazole	bobwhite quail	2223	*Leiostomus xanthurus*	2.6	*Daphnia magna*	10.2	*Skeletonema costatum/20*	0.02	>100	686
propineb	japonese quail	>5000	rainbow trout/96	0.4	*Daphnia magna*	4.7	*/96h*	2.7	>70	700
pyrimethanil	mallard ducks	>2000	rainbow trout/96	10.6	***Daphnia magna***	2.9	*/96*	1.2	>100	625
quinoxyfen	bobwhite quail	>2250	rainbow trout/96	0.27	*Daphnia magna*	0.08	*Selenastrum capricornutum72*	0.058	>100	**>923**
sulfur	bobwhite quail	>5000 ppm	not toxic	180	***Daphnia magna***	5000	*Ankistrodesmus bibraianus/72*	>232	not toxic	**2000**
tebuconazole	bobwhite quail	1988	rainbow trout/96	4.4	***Daphnia magna***	2.79	*Selenastrum capricornutum/72*	3.8	83	1381
tetraconazole	bobwhite quail	>63	rainbow trout/96	5.1	***Daphnia magna***	3	***Ankistodesmus bibaiamus***	2.4	>130	1000
thiabendazole	bobwhite quail	>2250	rainbow trout/96	0.55	*Daphnia magna*	0.81	*Selenastrum capricornutum/96*	9	n tóxico	>500
thiram	starlings	>100	sunfish/96	0.0445	-		*72h*	0.065	>2000/oral	540
tolylfluanid	bobwhite quail	>2000	rainbow trout/96	0.045	-		*Scenedesmus subspicatus/72*	>1.0	>197/oral	>1000
trifloxystrobin	bobwhite quail	>2000	rainbow trout/96	0.015	***Daphnia magna***	0.011	*Scenedesmus subspicatus*	0.0053	>200	>1000
ziram	bobwhite quail	97	rainbow trout/96	1.9	*Daphnia magna*	0.048	*not toxic*		>100	190
zoxamide	bobwhite quail	>2000	rainbow trout/96	160 ug/l	*Daphnia magna*	>780 ug/l	*Scenedesmus subspicatus*	0.011	100 /contact	>1070

	Birds		Fish		*Aquatic invertebrates*		Algae		Honeybees	Earthworms
Active substance HERBICIDES	Specie	Acute LD50 mg/Kg	Species/ Test duration (h)	Acute LC_{50} (mg/l)	Species/ Test duration (h)	Chronic (mg/l)	Species/ Test duration (h)	Acute EC_{50} (mg/l)	LD_{50} (µg/honeybee)	Acute LC_{50} mg/Kg soil
amitrole	mallard ducks	>2150	rainbow trout/96	>1000	*Daphnia magna/48*	6.1	*Scenedesmus subspicatus/72*	2.3	>150	>488
benoxacor	bobwhite quail	2000	trout (96h)	2.4	***Daphnia magna***	**4.8**	*Scenedesmus subspicatus/72*	0.63	>100	>1000
bentazone	bobwhite quail	1140	trout and sunfish	>100	*Daphnia magna*	125	*Green algae*	47.3	>100	870
bromoxinil	bobwhite quail	217	sun fish (96h)	29.2	***Daphnia magna***	**12.5**	*Scenedesmus subspicatus/96*	44	5	45
cycloxydim	bobwhite quail	>2000	sunfish/96	>100	*Daphnia magna*	**70.8**	*Pseudokirchneriella subcapitata*	44.9	>100	>1000
dicamba	mallard ducks	2000	rainbow trout/96h	135	***Daphnia magna***	110.7	*Unknown species*	>250	>100	1000
diflufenican	bobwhite quail	>2150	carp	98,5 ug/l	*Daphnia magna*	10	*Unknown species*	10	**100**	**500**
diquat	*Anas platyrhynchos*	83	*Salmonidae*	21	*Daphnia magna*	1.2	*Raphidocelis subcapitata*	0.011	**13**	**130**
diuron	bobwhite quail	1104	minnows(96 h)	6.7	*Daphnia magna/48*	1.4	*Selenastrum capricornutum/120*	0.022	100	>400
fluazifop-P-butyl	mallard ducks	>3500	rainbow trout/96	1.3	*Daphnia magna/48*	>1,0	*Navicula pelliculosa/72*	0.51	>200	>1000
flufenacet	***Colinus virginianus***	**1608**	***Oncorhynchus mykiss***	**0.2**	***Daphnia magna***	**30.9**	***Raphidocelis subcapitata/72***	**0.00204**	**170**	**219**
foramsulfuron	***Colinus virginianus***	**2000**	***Salmonidae***	**100**	***Daphnia magna***	**100**	***Anabaena flos-aquae***	**3.3**	**226.3**	**453**
forchlorfenuron										
glufosinate-ammonium	**bobwhite quail**	**2000**	rainbow trout/96	710	***Daphnia magna***	***688***	***Scenedesmus quadricauda***	**46.5**	>100	**1000**
glyphosate	bobwhite quail	>3851	trout/96	86	***Daphnia magna***	**11**	*Skeletonema costatum/96h*	1.3	>100 oral	**480**
iodosulforon-methyl-sodium	***Colinus virginianus***	**2000**	***Lepomis macrochirus***	**100**	***Daphnia magna***	**100**	***Pseudokirchneriella subcapitata/72***	**0.07**	**80**	**1000**
isoxaben	bobwhite quail	>2000	bluegill sunfish/96	>1,1	***Daphnia magna***	**1.3**	*Selenastrum capricornutum/14*	>1,4	>100	**500**
linuron	***Colinus virginianus***	**314**	***Salmonidae***	**3.15**	***Daphnia magna***	**0.31**	***Raphidocelis subcapitata/72***	**0.016**	**160**	**>1000**
mesotrione	***Colinus virginianus***	**2000**	***Lepomis macrochirus***	**120**	***Daphnia magna***	**622**	***Raphidocelis subcapitata***	**3.5**	**11**	**437.7**
metribuzin	bobwhite quail	164	rainbow trout/96	74.6	***Daphnia magna***	**49**	*Scenedesmus subspicatus*	0.021	35	331.8
metsulfuron-methyl										
nicosulfuron	***Colinus virginianus***	**2000**	***Oncorhynchus mykiss***	**65.7**	***Daphnia magna***	**90**	***Anabaena flos-aquae***	**7.8**	**76**	**1000**
oxifluorfen	bobwhite quail	>2150	bluegill sunfish/96	0.2	***Daphnia magna/48***	**0.72**	*Pseudokirchneriella subcapitata*	2	**100 /contact**	>1000
paraquat dichloride	bobwhite quail	175	rainbow trout/96	26	***Daphnia magna***	**6.1**	*Raphidocelis subcapitata*	0.00023	**36**	>1380
pendimethalin	***Anas platyrhynchos***	**1421**	***Oncorhynchus mykiss***	**0.138**	***Daphnia magna***	**0.28**	***Raphidocelis subcapitata***	**0.006**	**100**	**1000**
profoxydim	***Colinus virginianus***	**2000**	***Oncorhynchus mykiss***	15	***Daphnia magna***	**18.1**	***Anabaena flos-aquae***	**33**	**200**	**1000**
propaquizafop	***Colinus virginianus***	**2000**	***Oncorhynchus mykiss***	**1.19**	***Daphnia magna***	**2.1**	***Raphidocelis subcapitata***	**2.1**	**20**	**1000**
prosulfuron	***Anas platyrhynchos***	**1000**	***Oncorhynchus mykiss***	**160**	**-**		**-**		**-**	**>1000**
quinclorac										
quizalofop-P -ethyl	mallard ducks and	>2000	***Oncorhynchus mykiss***	>0,5	***Daphnia magna***	**0.29**	***Raphidocelis subcapitata***	**0.021**	**100 /contact**	>1000
rimsulfuron	mallard ducks	>2000	***Oncorhynchus mykiss***	110	***Daphnia magna***	**360**	***Raphidocellis subcapitata/120***	**0.029**	**100 /contact**	>1000
s- metolachlor	*Anas platyrhynchos*	2510	***Oncorhynchus mykiss***	1.23			***Raphidocellis subcapitata***	**0.008**	**85**	570
sulcotrione	mallard ducks	>1350	***Oncorhynchus mykiss***	240	*Daphnia magna/48*	>848	*Selenastrum capricornutum/96*	**3.5**	>50	>1000
terbuthylazine	mallard ducks and	>1000	***Oncorhynchus mykiss***	3.8	***Daphnia magna***	21.2	*Scenedesmus subspicatus/72*	0.016	>100	>200
tribenuron-methyl	***Colinus virginianus***	**2250**	***Oncorhynchus mykiss***	**738**	***Daphnia magna***	**894**	**-**		**9.1**	**1000**
trifluralin	bobwhite quail	>2000	***Oncorhynchus mykiss***	0.088	***Daphnia magna***	0.245	*Selenastrum capricornutum/7*	12.2	>100	>1000

bold- values from "Footprint" online database

- no available data

LD_{50}- Median lethal dose; LC_{50}- Median lethal concentration

ANNEX D

***Table D.1**. Predicted environmental distribution (PED) to the registered pesticides selected, obtained by the application of Mackay fugacity model (Level I)*

Active substance	PED's (%)						
INSECTICIDES	WATER	SOIL	AIR	SEDIMENT	SUSPENDED SOLIDS	AQUATIC BIOTHIC	AEROSSOL
abamectin	4.17	92.9	4.00E-01	2.06	6.50E-02	5.20E-03	4.40E-01
acetamiprid	99.4	0.556	1.10E-06	0.0123	3.90E-04	3.10E-05	1.10E-03
acrinathrin	0.0383	10.5	3.80E-04	0.234	7.30E-03	4.90E-04	8.90E+01
alpha-cypermethrin	0.0127	97.7	6.30E-03	2.17	6.80E-02	5.50E-03	3.00E-03
beta-cyfluthrin	94.8	0.344	3.90E-07	7.65E-03	6.77E-02	1.00E-02	
bifenthrin	0.11	97.1	2.30E-01	2.16	6.70E-02	5.50E-03	4.10E-04
buprofezin	1.72	96.1	1.20E-02	2.13	6.70E-02	5.40E-03	9.40E-07
carbaryl	94	5.89	1.30E-03	0.131	4.10E-03	3.30E-04	8.60E-05
carbofuran	97.1	2.85	9.90E-04	0.0633	2.00E-03	1.60E-04	7.50E-05
chlorpyrifos	2.15	95.4	3.00E-01	2.12	6.60E-02	5.40E-03	7.80E-03
chlorpyrifos-methyl	5.94	91.5	4.50E-01	2.03	6.40E-02	5.20E-03	2.50E-04
clofentezine	8.01	89.4	2.80E-02	1.99	6.20E-02	5.00E-03	5.50E-01
cypermethrin	0.0277	97.7	1.20E-04	2.17	6.80E-02	5.50E-03	2.10E-02
cyromazine	99.9	0.0769	1.20E-06	0.00171	5.30E-05	4.30E-06	2.30E-03
deltamethrin	0.0291	2.40E-01	1.90E-04	5.30E-03	1.70E-04	1.10E-05	1.00E+02
dicofol	5.24	92.6	2.60E-02	2.06	6.40E-02	5.20E-03	1.70E-04
diflubenzuron	12.4	85.6	1.20E-03	1.9	5.90E-02	4.80E-03	9.10E-04
dimethoate	99.5	0.446	5.00E-04	0.00991	3.10E-04	2.50E-05	3.70E-03
esfenvalerate	0.0664	97.6	5.70E-04	2.17	6.80E-02	5.50E-03	1.00E-05
fenamiphos	35.6	62.9	6.70E-03	1.4	4.40E-02	3.60E-03	9.90E-04
fenazaquin	0.34	97.4	3.30E-04	2.16	6.80E-02	5.50E-03	1.30E-02
fenoxycarb	8.59	89.4	5.80E-05	1.99	6.20E-02	5.00E-03	6.10E-04
flufenoxuron	9.16	81.1	1.40E-05	1.8	5.60E-02	9.20E-03	7.90E+00
imidacloprid	99.7	3.28E-01	3.50E-09	7.29E-03	2.30E-04	3.70E-05	5.90E-05
indoxacarb	2.41	95.4	2.97E-05	2.12	0.0662	0.0108	0.0294
lambda-cyhalothrin	0.011	97.7	4.10E-05	2.17	6.80E-02	5.50E-03	1.20E-02
lufenuron	0.83	96.9	5.80E-03	2.15	6.70E-02	5.50E-03	4.00E-07
malathion	66.1	32.9	1.60E-01	7.32E-01	2.30E-02	1.90E-03	2.80E-04
methidation	87.4	12.3	6.80E-03	2.73E-01	8.50E-03	6.90E-04	1.10E-02
methiocarb,	47.9	51	1.20E-03	1.13	3.50E-02	2.90E-03	9.90E-04
methomyl	99.9	0.11	4.10E-05	0.00244	7.60E-05	6.20E-06	1.80E-06
methoxyfenozide	18	80.1	6.10E-04	1.78	5.60E-02	4.50E-03	3.10E-04
oxamyl	100	0.0321	8.20E-06	0.000714	2.20E-05	1.80E-06	7.60E-04
phosalone	9.73	88.2	1.40E-02	1.96	6.10E-02	5.00E-03	1.60E-02
phosmet	55.3	43.7	9.40E-03	9.70E-01	3.00E-02	2.50E-03	4.70E-04
pirimicarb	95.7	4.25	6.20E-04	9.43E-02	3.00E-03	2.40E-04	1.60E-05
pymetrozine	99.9	0.0585	6.10E-05	1.30E-03	4.10E-05	3.30E-06	1.60E-05
spinosad	9.94	88	1.90E-07	1.96	6.10E-02	5.00E-03	1.50E-04
spirodiclofen	0.175	97.6	8.80E-04	2.17	6.80E-02	5.50E-03	6.50E-06
tau-fluvalinate	2.85	46	2.40E-05	1.02	3.20E-02	5.20E-03	5.00E+01
tebufenozide	5.84	92	7.90E-05	2.05	6.40E-02	5.20E-03	1.20E-03
teflubenzuron	5.19	92	7.40E-03	2.04	6.40E-02	1.00E-02	6.90E-01
thiacloprid	42.4	56.3	3.60E-09	1.25	3.90E-02	7.70E-05	9.70E-05
thiamethoxam	99.9	0.0656	9.60E-09	0.00146	4.60E-05	7.40E-06	1.10E-05
trichlorfon	99.8	2.38E-01	9.20E-06	5.28E-03	1.70E-04	1.30E-05	1.40E-06

Active substance	PED's (%)						
FUNGICIDES	WATER	SOIL	AIR	SEDIMENT	SUSPENDED SOLIDS	AQUATIC BIOTHIC	AEROSSOL
azoxystrobin	46.1	49.8	6.90E-08	1.11	2.40E-04	1.60E-05	4.90E+00
benalaxyl	24.1	74.1	3.70E-02	1.65	5.20E-02	4.20E-03	7.30E-03
benalaxyl-M	19.1	79.1	2.30E-03	1.76	5.50E-02	4.50E-03	1.30E-03
bitertanol	94.8	0.344	3.90E-07	0.00765	2.40E-04	1.60E-05	4.90E+00
bupirimate	12.2	85.8	6.10E-03	1.91	6.00E-02	4.90E-03	3.60E-03
captan	62.7	35	1.50E+00	0.778	2.40E-02	2.00E-03	1.60E-02
carbendazim	97.1	2.78	7.10E-02	6.20E-02	1.90E-03	1.60E-04	7.90E-05
chlorothalonil	56.9	41.9	2.90E-01	0.931	2.90E-02	2.40E-03	2.10E-03
cyazofamid	40.9	57.4	3.30E-01	1.27	4.00E-02	3.20E-03	5.10E-03
cymoxanil	99.6	0.343	7.80E-04	7.60E-03	2.40E-04	1.90E-05	3.90E-09
cyprodinil	9.94	88	1.80E-02	1.96	6.10E-02	5.00E-03	1.20E-03
difenoconazole	4.21	93.6	7.70E-07	2.08	6.50E-02	5.30E-03	6.60E-04
dimethomorph	72.1	27.2	1.10E-04	0.606	1.90E-02	1.50E-03	3.20E-33
dinocap	3.08	94.6	5.10E-03	2.1	6.60E-02	5.30E-03	1.70E-04
dithianon	41	57.6	4.80E-05	1.28	4.00E-02	6.50E-03	2.30E-02
dodine	96.1	3.8	9.00E-05	8.50E-02	2.60E-03	2.20E-04	1.30E-05
famoxadone	2.41	95.4	2.30E-03	2.12	6.60E-02	5.40E-03	9.80E-06
fenamidone	63.6	35.6	1.80E-04	0.79	2.50E-02	2.00E-03	2.10E-03
fenarimol	18.4	79.8	5.90E-03	1.77	5.50E-02	4.50E-03	1.10E-03
fenbuconazole	39.3	59.1	2.50E-01	1.31	4.10E-02	3.30E-03	1.80E-06
fluazinam	5.64	62.9	3.00E+01	1.4	4.40E-02	3.60E-03	5.20E-02
fludioxonil	7.73E+00	9.02E+01	8.50E-05	2.00E+00	6.30E-02	5.10E-03	4.10E-04
fluquinconazole	34.1	64.4	1.50E-05	1.43	4.50E-02	5.90E-03	5.00E-03
flusilazole	16.7	81.4	7.80E-04	1.81	5.70E-02	4.60E-03	2.70E-04
folpet	46.1	52.6	7.40E-02	1.17	3.70E-02	3.00E-03	1.10E-02
fosetyl-aluminium	100	3.53E-04	6.50E-09	7.80E-06	2.50E-07	2.00E-08	6.10E-02
imazalil	52.3	46.3	2.30E-01	1.03	3.20E-02	2.60E-03	8.30E-02
iprovalicarb 617	42.1	56.5	1.40E-05	1.26	3.90E-02	3.20E-03	1.10E-03
mancozeb	99.8	1.61E-01	1.20E-02	3.60E-03	1.10E-04	9.10E-06	1.10E-04
metalaxyl-M	95.6	4.34	7.00E-04	9.60E-02	3.00E-03	2.50E-04	1.10E-03
myclobutanil	55.9	43.1	5.00E-03	9.60E-01	3.00E-02	2.40E-03	5.30E-04
penconazole	17.4	80.8	2.40E-03	1.79	5.60E-02	4.60E-03	3.10E-05
procymidone	41.9	51.2	5.70E+00	1.14	3.60E-02	2.90E-03	1.70E-03
propamocarb hydrochloride	100	5.46E-03	3.50E-07	1.20E-04	3.80E-06	3.10E-07	4.00E-07
propiconazole	17.4	80.8	3.30E-04	1.79	5.60E-02	4.60E-03	1.50E-03
propineb	99.9	4.87E-02	7.10E-07	1.10E-03	3.40E-05	5.50E-06	2.60E-02
pyrimethanil	61.4	37.6	4.60E-02	8.40E-01	2.60E-02	2.10E-03	1.40E-06
quinoxyfen	2.36	95.4	3.80E-02	2.12	6.60E-02	5.40E-03	5.10E-02
tebuconazole	18	80.1	5.40E-05	1.78	5.60E-02	4.50E-03	5.30E-04
tetraconazole	23.3	75	2.10E-03	1.67	5.20E-02	4.20E-03	1.40E-03
thiabendazole	81.8	17.8	5.20E-05	4.00E-01	1.20E-02	1.00E-03	2.20E-05
thiram	94.8	4.51	6.00E-01	1.00E-01	3.10E-03	2.60E-04	1.40E-03
tolylfluanid	12.2	85.8	1.90E-02	1.91	6.00E-02	1.80E+00	2.10E-02
trifloxystrobin	3.37	94.5	1.60E-06	2.1	6.60E-02	5.30E-03	1.60E-05
ziram	98.5	1.48	6.20E-04	3.30E-02	1.00E-03	8.40E-05	4.00E-04
zoxamide	16.1	82	1.60E-02	1.82	5.70E-02	4.60E-03	7.60E-03

Active substance	PED's (%)						
HERBICIDES	WATER	SOIL	AIR	SEDIMENT	SUSPENDED SOLIDS	AQUATIC BIOTHIC	AEROSSOL
amitrole	4.01	93.8	1.50E-08	2.09	6.50E-02	4.30E-03	7.80E-04
benoxacor	73.4	25.9	1.20E-01	0.575	0.018	1.46E-03	
bromoxynil	99	9.60E-01	1.10E-05	2.10E-02	6.70E-04	5.40E-05	
cycloxydim	98	1.99	1.20E-03	4.40E-02	1.40E-03	1.10E-04	
dicamba	100	1.20E-03	3.00E-04	3.30E-05	8.10E-07	6.60E-08	
diflufenican	1.37	96.4	9.40E-03	2.14	6.70E-02	5.40E-03	5.50E-03
diuron	60.9	38.2	8.60E-05	8.50E-01	2.70E-02	2.20E-03	
fluazifop-P-butyl	1.22	96.5	2.30E-02	2.15	6.70E-02	5.50E-03	
flufenacet	41.1	57.6	4.30E-03	1.28	4.00E-02	3.30E-03	1.70E-03
foramsulfuron	92.3	7.57	1.10E-10	9.70E-01	5.30E-03	1.50E-06	4.90E-06
forchlorfenuron	41	57.6	1.10E-07	1.28	4.00E-02	6.50E-03	1.10E-05
glufosinate-ammonium	99.9	1.10E-01	3.00E-07	2.50E-03	7.70E-05	6.30E-06	1.90E-05
glyphosate	100	8.86E-05	2.40E-07	1.97E-06	6.20E-08	5.00E-09	6.20E-08
iodosulforon-methyl-sodium	100	0.0177	2.90E-09	0.000393	1.30E-05	1.00E-06	2.50E-06
isoxaben	11.2	86.8	3.00E-04	1.93	6.00E-02	4.90E-03	1.70E-03
linuron	52.5	46.5	2.10E-03	1.03	3.20E-02	2.60E-03	2.30E-04
mesotrione	100	0.0088	1.80E-05	0.000197	6.20E-06	5.00E-07	1.30E-05
metribuzin	96.5	3.4	2.30E-04	7.60E-02	2.40E-03	1.90E-04	4.20E-05
metsulfuron-methyl	99.9	9.20E-02	9.20E-10	2.10E-03	6.40E-05	1.00E-05	1.30E-05
nicosulfuron	100	1.40E-03	3.00E-10	3.12E-05	9.80E-07	1.60E-07	1.40E-06
oxifluorfen	3.6	94.1	6.20E-02	2.09	6.50E-02	5.30E-03	2.20E-05
pendimethalin	0.69	96.8	2.30E-01	2.15	6.70E-02	5.50E-03	8.10E-04
propaquizafop	1.8	96	3.40E-08	2.13	6.70E-02	1.10E-02	3.20E-03
prossulforão	99.9	5.50E-02	1.00E-03	1.20E-03	1.89E-03	1.54E-04	6.00E-05
quinclorac	99.2	0.016	7.60E-01	0.000355	1.10E-05	9.00E-07	2.50E-02
quizalofop-P -ethyl	2.64	95.2	3.60E-05	2.11	6.60E-02	5.40E-03	
rimsulfuron	100	3.00E-03	1.30E-03	6.67E-05	2.10E-06	1.70E-07	3.10E-03
s- metolachlor	49.6	49.3	2.20E-02	1.09	3.40E-02	2.80E-03	7.20E-04
sulcotrione	99.9	8.90E-02	2.00E-04	0.00197	6.10E-05	5.00E-06	0.000311
terbuthylazine	40.5	58.1	3.40E-02	1.29	4.00E-02	3.30E-03	6.90E-04
tribenuron-methyl	99.5	5.30E-01	2.10E-07	1.20E-02	3.70E-04	6.00E-05	2.90E-05
trifluralin	1.55	92.9	3.40E+00	2.06	6.50E-02	5.30E-03	3.00E-02

ANNEX E

Analytical methodology: SPME-GC-MS

Solid phase microextraction (SPME) and dosage by Gas chromatography-mass spectrometry (GC-MS) was performed in the Ecotoxicology Laboratory of DPFF/ISA.

A standard procedure is described above:

I. Solid phase microextraction (SPME)

1º - Sample preparation

- Weight 1g of NaCl and add to 10ml of water sample (or pattern solutions to determine the calibration curves. Place the 10ml glass, containing the water sample, in the GC injector (Combi Pal CTC Analytics AG model);

2º - Extraction

- Water samples' pesticides residues extraction, in the injector port, resorting to fibres (of SPME) with Carbowax/divinylbenzene (CW/DVB) coating.
- Absorption of analytes: by dipping the SPME fibre in the sample, during 60 minutes, with stirring with the energy of 250 rpm.

II. Desorption, dosage and quantification by gaseous chromatography coupled with mass-spectrometry (GC)

Gas Cromatograph "Varian Chrompack CP-3800" coupled with a mass-spectrometer "Saturn 2000 GC/MS" by Varian was used.

The chromatographic conditions were:

- GC Injector: split/slitless, with valve opening past 5 minutes;
- GC Injector temperature: 240°C;
- Column: J&W DB-5MS 30m x 0,25mm Low Bleed/MS, with 0,25µm of film thickness;
- Oven's range of temperatures: 50°C in the beginning (1 minute), range of 10°C/min till 170°C, range of 1°C/min till 180°C, range of 5°C/min till 220°C (6 minutes), range of 15°C/min till 240°C/min (4 minutes);
- Carrier Gas: Helium C-60 (GASIN, Perafita, Porto);
- Flow of the carrier gas: 12 Psi (Ψ)
- Detector: Ion Trap;

- Ionization mode: by Electronic Impact (EI); spectrum obtained at 70 eV, in full scan from m/z 70 to m/z 350;
- Axial modulation voltage: 4.0;
- Temperature of the Manifold: 40ºC;
- Temperature of the Ion Trap: 190°C;
- Transfer line temperature: 230°C.

Compounds from the GC column were identified comparing their retention times and mass spectrum with the referential retention time and mass spectrum – resulted from the analysis of the standard solution.

The main ionic fragments (m/z, i.e., mass/charge) were considered for the identification and quantification of pesticides. Dosage was based on the peaks area achieved, and through the curve calibration, obtained from standard solutions containing the pesticides mixture in question, of 0.05µm/L, 0.1µm/L, 0.25µm/L, 0.5µm/L, 1µm/L e 5µm/L.

ANNEX F

Procedure for the growth inhibition test using the freshwater algae *Pseudokirchneriella subcapitata* based upon the OECD (1984) and EEC (1989) guidelines

A brief sum up of the main steps to accomplish the test is described above:

1- Preparation of the algal inoculum;
2- Preparation of test solutions
3- Dispensing test solutions, algal inoculum, and nutrient spike to the microplate;
4- Test observations and measurements;
5- Test endpoints and calculations/ Data analysis.

1- Algal inoculums preparation

Pseudokirchneriella subcapitata was maintained in 100-ml nonaxenic batch cultures, with Woods Hole MBL growth medium, 19 to 21 ºC under continuous cool-white fluorescent illumination (100 µE/m^2/s) (Figure H2).

The algal inoculum is composed of *P. subcapitata* cells harvested from a liquid stock algal culture (that is 4 to 7 days old and in a logarithmic phase of growth). The harvest cells are centrifuged at 3500 rpm during 5 min, the supernatant discarded and the cells were resuspended. It is imperative that the initial cell density for the test presents a value of 10000cells/mL. For this purpose the cells concentrations were determined using a Neubaeur chamber.

2- Test solutions preparation

Accordingly to Environment Canada (1992) guideline , it is recommended to include a concentration that has no effect on algal-cell yield, and other that completely inhibits algal growth, and two concentrations each, i.e., above and below the IC50 value. Therefore, these ranges of dilution gradients concentrations series, 6.25, 12.5, 25, 50 and 100% in AlmondaR-2 and D20-1 samples, were established (Table 1.H).

The dilution factor 2 was used to prepare the geometric series of test concentrations of both samples, resulting in a total of 40 ml of sample for each.

Table 1.F. P.subcapitata microplate technique dilution gradient

Dilution (%)	Sample Quantity(mL)	MBL Quantity (mL)
100	40	-----
50	20	20
25	10	30
12.5	5	35
6.25	2.5	37.5

To ensure the homogeneity, the two samples designed as DV an QB were vigorously shaken and filtered through a preconditioned membrane of 0.45 µm pore diameter.

The MBL medium was diluted 2.5 times to be used as the control and dilution medium according to the required N/P ratios.

3- Dispensing test solutions, algal inoculums, and nutrient spike to the microplate

With the multichannel pipette 2 ml of reagent water were dispensed to each 16 peripheral wells of the microplate. As a result the range of dilution gradients were excluded - always starting with the lowest concentration and ending with the highest concentration of the test dilution, i.e., the lowest dilution. Following pipetting 2 ml of MBL into each 6 wells control was performed.

To accurate conditions for the algal microplate toxicity test, in the 72 h test, the temperature and lighting were verified. While testing it is recommended to keep the algae in suspension by transfer of CO_2. For this exact purpose, a multichannel micropipette it was used - first the contents were drawn from the wells and then back into the wells.

Three 50-ml replicate cultures of each test dilution and six of the control were set up and inoculated with a 1 ml algal inoculum, so that the initial cell concentration was 10^4 cells/mL (Figure H1).

4- Test observations and measurements

pH and conductivity (µS/cm) were measured at the beginning of the test and after 72 hours, and should not deviate by more than one unit during the test (Environment Canada, 1992).

At the end of the 72 h exposure, the mean specific growth rate per day was estimated. Initial and final cell densities were counted on well-mixed aliquots of each replicate under a microscope (400× magnification), using a Neubauer chamber (American Optical, Buffalo, NY, USA).

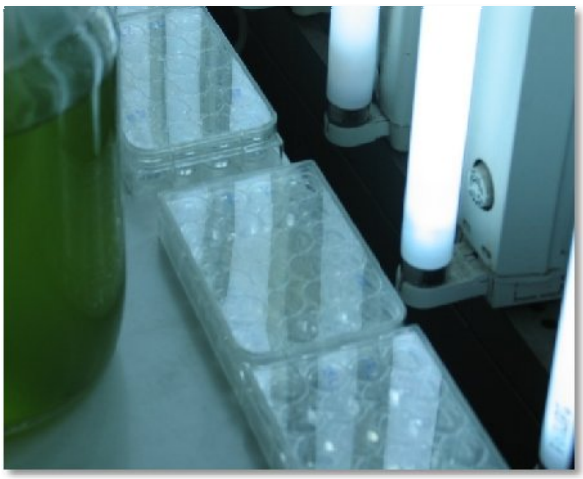
The conditions for the legitimacy of the test are, according to the protocols USEPA, 1989; OECD, 1984; Environment Canada, 1992/1997):

(1) The cell concentration in the control cultures should have increased by a factor of at least 16 within three days;

(2) Disappearance of the test substance from water into the biomass does not necessarily invalidate the test;

(3) Homogeneity must be demonstrated for the standard control wells, among the measurements or photometric estimates off all yields. For a valid test, the coefficient of variation must not exceed 20%.

The growth medium used in this method, as mentioned above, Woods Hole MBL, consists of five stock nutrients solutions and reagent water. In some cases the inhibition of growth can be attributed to the nutrient deficiency inherent to the test solution (Environment Canada, 1992).



PICTURE F.1: Microplates in incubation

PICTURE F.2: Algae stock culture

ANNEX G

Procedure for the growth inhibition test using the fresh water algae *Pseudokirchneriella subcapitata* (ALGALTOXKIT)

The validation of this method depends on the algae number (or algae biomass) in the control that must increase at least a factor of 67 times at the end of 72 h. Also the pH control should not oscillate more than 1.5 units since the beginning of the test.

The inhibition of algae growth in relation to the control is determined by reading of the Optical Density (OD) of algae suspensions in spectrophotometer every day, i.e., after 24h and 72h. The cells were incubated for a period of three days at 8000 2000 lux and 23 2 ºC.

For this test the following steps were taken:

1. For the correction of the turbidity and the intense color, the samples were filtered in vacuum with a 0.45 µm filter, before starting the tests. Subsequently the filtered samples were put in properly identified glass bottles.

2. Preparation of the Algae culture medium**:** a 1L volumetric flask was filled with 800 mL of demineralized water. Then it were added commercial nutrients solutions previously prepared (MicroBioTests): a full vial content of nutrient A (10 mL) and 1mL of the nutrients B, C and D. At last it was added up to 1L of demineralized water, and the solution was shaken. After, the adjustment of the pH to the value 8 0.2 was made using HCL or NaOH 1M.

3. Demobilization of Algae: the liquid medium, existing in the centrifugal tube that contains the algae, was discarded and 5mL of the dissolvent medium of the matrix was added. The tube was vigorously shaken to dissolve the matrix. The content of the tube was centrifuged for 10 min at 3000 rpm and the supernatant was discarded.

4. 5 ml of water was added and a second centrifugation took place under the same conditions, and the supernatant discarded.

5. Preparation of the concentrate algae inoculums : the content of the centrifuge was transferred to a 25 mL flask and the necessary volume was obtained with the addition of the culture medium of algae.

6. A innoculum of 10^6 cel/ml was obtained after several measurements of the optical readings.

For statistically acceptable evaluation, each concentration and control sample was prepared in triplicate. Then, three cells of 10 cm of the spectrophotometer were used, respectively two “cells calibration” and other as “stock cell”, and filled with 25 mL of the algae culture, covered and hit the zero to 670nm (self zero).

To correct the turbidity and the color of samples, they were filtered in vacuum with a 0.45 µm filter, before tested. The filtered samples were putted in properly identified glass bottles.

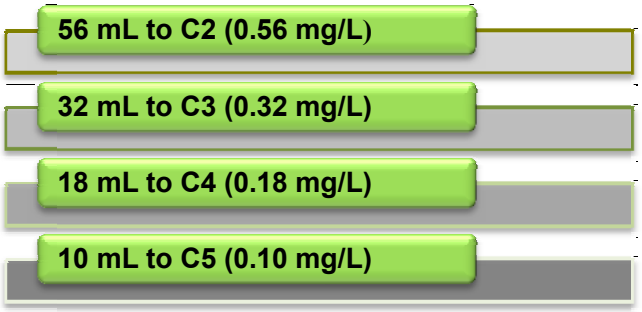
The next step consisted in transfer the samples to the reading cells. Two replicates of each one of the study samples designed as AlmondaR-1, ALMONDAR-2, D_{20}-1 AND D_{20}-2 (and each one was collected in 4 different dates) and two replicates for the three controls were prepared. For each one of the test cells were putted 25 mL of sample/control medium from each corresponding flask.

After the immobilization of the algae from the beads, 1 mL of a suspension corresponding to 10^6cells/mL was used in each non-diluted sample and controls to obtained algae concentration of $1x10^4$cells/mL.

The cells were incubated during the test at 8000 2000 lux and 23 2 ºC and putted in a tray at random to ensure that all of them have the same incubation conditions.

As recommended by ISO/DIS 8692 has been made the preparation of the reference sample (using potassium dichromate ($K_2Cr_2O_7$)) to confirm the physiological conditions of the used organisms. For this it was prepared a dilution series of 1 mg/L to 0.1 mg/L of potassium dichromate, first was placed potassium dichromate with a concentration of 1 g/L in a 1L volumetric flask, and it was filled with demineralized water to obtain thereby the stock solution. Next 8 volumetric flask of a 100 mL and labeled from C0 to C5, identifying two of them with C1 and the 8th like “stock 2” were prepared.

It was transferred 1 mL of the stock solution for the stock 2 flask and added up to 100 mL with the algae culture. A solution with final concentration of 10 mg/L was obtained. After, was transferred 10 mL of stock 2 for both C1 flask added up to 100 mL with algae culture, is obtained a final concentration of 1 mg/L in both flask.Then were retired from C1 the following volumes and placed in their respective flasks:



All flasks were fulfilled with100 mL of algae culture (except the flask C1).

Finally it was added 0.84 mL of algae inoculate with 1x106cells/mL to the flask C1 and 1mL to the others, reaching algae density of 1x104cells/mL in all flaks.

The inhibition of algae growth in relation to the control is determined by reading the absorbance (with a UV spectrophotometer) of algae suspensions in spectrophotometer at 0h, 48h and 72h after the test beginning.

Before each spectrophotometer reading session, the verification procedure of the apparatus was carried out

Before absorbance reading, all cells were shaken and during the next 10 seconds the reading was done (while the algae remained suspended), being all values registered.

The conductivity (µS/cm) and dissolved oxygen (%) were measured and the validity conditions were verified. At the end of 72-h de growth inhibition per day was calculated.

ANNEX H

Procedure for the immobilization rate using the *Daphnia magna:* acute toxicity test

This is a 48-h static test, based on the norm ISO (International Standard Organiztion) – ISO/DIS 6431 (1996), without replacement of solutions, performed by resorting to young *Daphnia magna* obtained by dormant *Daphnia magna* eggs eclosion (Daphtoxkit F^{TM}).

For this test, dormant structures from daphnia (cists) are used, covered by an quitinosa capsule, the "ephippium", that besides giving protection to the daphnia it also keeps its viability during the years (SOP, 1998). For this test, death is the endpoint for *D. Magna.*

Turbidity, colour, salinity, pH and the oxygen content can interfere with the response of the organisms.

For the tests were considered two different procedures, respectively:

- The first test procedure, uses a single concentration, in this case 100%; the endpoint for this test is the mortality percentage at 48 hours, reported for each sample. As immobilization is the sole biological effect observed, as a consequence, the test's endpoint is the percentage of immobilization at 48 hours.

- The second procedure, is use to estimate the median lethal concentration (LC_{50}), or if necessary, the median effective concentrations for immobilization (EC_{50}) (i.e., it determines the degree of toxicity using several concentrations including full strength) (Environment Canada, 2000). For this test, five concentrations were used; the range of dilutions was 6.25, 12.5, 25, 50 and 100%.

The eclosion of the ephipias (dormant *Daphnia magna* eggs) took place after 72 hours, at a temperature of 21 ºC ± 2ºC and continuous light intensity of 6000 ± 1000 lux.

The validation criterion for this test, is that the mortality in the control, which is translated in the immobilization of the organisms, should not exceed 10% (Environment Canada, 2000).

In accordance to prior paragraphs, the following measures were taken in order to evaluate the toxicity of surface water samples:

1-Procedure for a single concentration tests to determinate the mortality rate of 48 h:

For each control chamber (test vessel) 10 ml of the medium culture and 10 ml of each sample concentrations were transferred to each chamber, identified as the samples AlmondaR-1, AlmondaR-2, D_{20}-1 and D_{20}-2.

For both sample and control there were 10 young daphnias, therefore 5 in each chamber, because at least ten daphnids per concentration are required for an LC50 test. The transfer of the daphnias to the plates consisted of two steps, primarily the transfer of the petri dish to the washing chambers of the plate; and then the transfer of the young from the washing chambers to the four chambers of the same line.

After the transfer, they were placed in the incubator with a temperature of 21± 2ºC. The numbers of dead daphnids in each vessel were registered after periods of 24 hours.

2-Procedure for a Multi- Concentration test to determine the 48-h LC_{50}:

Initially, the medium culture it was prepared, achieved by transfering of 1L of demineralized water to a 2L volumetric flask, to which it was added 10ml of $NaHCO_3$, $CaCl_2$, $MgSO_4$ and KCl solutions, in this particular order.

To fill de volumetric flask up to 2L demineralized water was added, which was stirred and kept in a fridge, in the dark.

After concluding the dilutions, 10mL of the culture medium were transferred to each of the control chambers (C) and 10mL of each sample concentrations to each chamber of the corresponding line by crescent order of concentration.

The transfer and the number of daphnias was the same as it was described in the previous procedure.

After the transfer, they were placed in the incubator at a temperature of 21± 2ºC.

The numbers of dead daphnids in each vessel were registered after periods of 24 hours.

The results are expressed in percentage of immobilization, i.e., the rate between the total numbers of immobile individuals and of organisms in the sample. The organisms are considered immobile when they continue immobile for a minimum period of 15 seconds.

ANNEX I

Procedure for the reproduction chronic test using the *Daphnia magna:* chronic toxicity

Organisms used for testing, were third-brood 6 to 24 h old neonates, obtained from cultures maintained at 20 to 22 ºC under a 14h:10h light: dark photoperiod. Reconstituted hard water culture was the medium (ASTM 2002a) supplemented with vitamins (7.5 µg/l of B1, 1 µg/l of B12 and 0.75 µg/l of biotin) and Marinure extract (Glenside, Stirling, UK) (7.5 ml/l of a suspension with an absorbance of 620 units at 400 nm). Cultures (25 and 15 daphnids/l up to the first brood and from there onwards, respectively) were fed daily with *P. subcapitata* (3 × 105 cells/ml) and the medium was renewed every other day.

The aim of the test, is to study the effect of pesticides on the reproductive output of *Daphnia magna* when compared to the controls, *i.e.,* enables the determination of the lowest-observed-effect concentration and hence the no-observe-effect concentration (NOEC) (OECD, 1998).

Aiming for this, the young female *Daphnia,* alive for less than 24 hours when the test began, was exposed to both superficial water samples with a wide range of concentrations, in a way that when the test ended the total number of living offspring produced per parent was assessed. Therefore, the remnants with dead juveniles were excluded from the analysis.

In order to carry out the toxicity test the following equipment was utilized:

- oxygen meter;
- pH-meter;
- conductivity- meter

The samples AlmondaR-2 and D_{20}-1 were subjected to a dilution gradient of 6.25, 12.5, 25, 50 and 100%, as recommended; still, if chronic toxicity tests had been previously performed with Daphnia, it would have facilitated the choice of a dilution range (OECD, 1998).

The culture medium was used as the control and dilution medium. Ten replicates were set up for each treatment, with 50 ml of the test solution and dilution medium, for semi-static tests, assuring the necessary conditions for LOEC/NOEC calculation (OECD, 1998).

On a daily basis, a diet for the animal parents was provided during the, consisting in *P. subcapitata*; further, the offspring produced by each parent was removed and counted daily, precisely to prevent them from consuming the food left for the adults (OECD, 1998).

The medium was renewed three times per week and also measured oxygen concentration, temperature, conductivity (µS/cm) and pH values all medium renewals, in fresh and old media. In accordance with the OECD guidelines, in order to validate a test, the following performance criteria should be found in the controls: (1) the mortality of the parent animals (female *Daphnia*) should not exceed 20% at the end of the test; (2) the mean number of offspring produced per parent animal surviving at the end of the test is should be ≥ 60.

ANNEX J

Procedure for the survival and growth test using the larvae of freshwater midges – *Chironomus riparius*

For this test, four-instar larvae were used, maintained in a transparent plastic cage (40×60×120 cm) sufficiently large to allow swarming and copulation of adults (OECD 2004), under a daily photoperiod of 14-h light and 10-h dark with 90- minutes dawn and dusk periods, at 20 to 22 ºC.

Four replicates with 3 organisms were set up for each test dilution and control, clearly coded to enable identification of the sample using 250 ml glass flasks, with 5.5 cm diameter.

Once, in a preliminary test, toxicity was not detected, only a 100% concentration was performed in order to assess the sediment toxicity.

The culture medium was used as the control and dilution medium. Two replicates were set up for test dilution and four to the control, with 45 g of sediment and 120 ml of medium in the replicates test and 50 of standard sediment and 120 ml of medium for the control vessels.

Vials were prepared 12 h prior to the beginning of the test and left with continuous aeration.

While adding the chironomids, aeration was stopped for a 30 min period, allowing larvae to burrow. After this short time, gentle aeration was restarted and kept continuously throughout the test. Food (ground Tetramin ®) was added in a single 1.5 mg/larva/day dose at day 0 and every 2 days henceforth. At day 7, organisms were collected by sieving the test sediment through a 500-µm mesh. The larvae recovered from each replicate flask were pooled and weighed after drying at 105 °C for 12 h in an oven (dry weight) and ignite in a muffle furnace at 450 °C for 6 h (ash weight). This allowed the calculation of the ash-free dry weight (AFDW), which was used as a measure of chironomid growth (biomass).

Mortality, pupation and number of emerged adults were also determined at the end of the test.

Conductivity, pH and oxygen concentration were measured at the beginning and end of the test for at least one test chamber representing each treatment.

For a valid test, the mean survival rate for midge larvae in control sediment must be ≥ 70% at the end of the test; and individual mean dry weight for replicate controls at test end must average > 0.5 mg per individual organism (OECD, 2004).

ANNEX K

***Table K.1**. Results from the PRIHS-1 index.*

			EARTHORMS			BENEFICIAL ARTHROPODS			MAMMALS				
Pesticides	MRA (g/ha)	PEC=MRA/750	LC_{50}	LC_{50}/PEC	SCORE (A)	LD_{50}	%Efeito (MRA/LD_{50})	SCORE (B)	LD_{50cut}	LD_{50der}/PEC	SCORE (C)	PRIHS-1	Final score
abamectin	13.5	0.02	28	1555.6	0	0.0022	6136.4	8	2000	111111.1	0	40	Medium
acetamiprid	100	0.13	9	67.5	2	14.5	6.9	1	2000	15000.0	0	16	Medium
acrinathrin	75	0.10	1000	10000.0	0	175	0.4	0	2000	20000.0	0	0	Negligible
alpha-cypermethrin	30	0.04	100	2500.0	0	0.059	508.5	4	2000	50000.0	0	20	Medium
amitrole	3600	4.80	488	101.7	1	150	24.0	2	2500	520.8	1	18	Medium
azoxystrobin	200	0.27	283	1061.3	0	25	8.0	1	2000	7500.0	0	5	Negligible
benalaxyl	160	0.21	180	843.8	1	100	1.6	1	5000	23437.5	0	11	Low
benalaxyl-M	100	0.13	472.7	3545.3	0	104	1.0	0	2000	15000.0	0	0	Negligible
benoxacor	75	0.10	1000	10000.0	0	100	0.8	0	2010	20100.0	0	0	Negligible
bentazone	1200	1.60	870	543.8	1	100	12.0	2	2500	1562.5	0	16	Low
beta-cyfluthrin	12.5	0.02	1000	60000.0	0	0.001	12500.0	8	5000	300000.0	0	40	Medium
bifenthrin	80	0.11	1000	9375.0	0	0.1	800.0	4	2000	18750.0	0	20	Medium
bitertanol	187.5	0.25	1000	4000.0	0	104.4	1.8	1	5000	20000.0	0	5	Negligible
bromoxinil	450	0.60	45	75.0	2	5	90.0	2	1000	1666.7	0	21	Medium
bupirimate	250	0.33	1000	3000.0	0	50	5.0	1	4800	14400.0	0	5	Negligible
buprofezin	125	0.17	500	3000.0	0	163.5	0.8	0	5000	30000.0	0	0	Negligible
captan	2000	2.67	519	194.6	1	100	20.0	2	4500	1687.5	0	16	Low
carbaryl	1000	1.33	106	79.5	2	1	1000.0	4	2000	1500.0	0	31	Medium
carbendazime	600	0.80	6	7.5	4	50	12.0	2	2000	2500.0	0	32	Medium
carbofuran	500	0.67	13	19.5	2	0.04	12500.0	8	2000	3000.0	0	51	High
chlorothalonil	1500	2.00	268.5	134.3	1	63	23.8	2	2000	1000.0	1	18	Medium
chlorpyrifos	960	1.28	210	164.1	1	0.059	16271.2	8	1250	976.6	1	48	High
chlorpyrifos-methyl	200	0.27	182	682.5	1	0.38	526.3	4	2000	7500.0	0	26	Medium
cimoxamil	120	0.16	2208	13800.0	0	25	4.8	1	2000	12500.0	0	5	Negligible
copper (hydroxide)	2750	3.67	667.3	182.0	1	44.5	61.8	2	2000	545.5	1	18	Medium
cyazofamid	80	0.11	1000	9375.0	0	151.7	0.5	0	2000	18750.0	0	0	Negligible
cyfluthrin	1250	1.67	1000	600.0	1	0.001	1250000.0	8	5000	3000.0	0	46	High
cymoxanil	400	0.53	1000	1875.0	0	100	4.0	1	2000	3750.0	0	5	Negligible
cypermethrin	100	0.13	100	750.0	1	0.035	2857.1	8	2460	18450.0	0	46	High
cyprodinil	300	0.40	192	480.0	1	100	3.0	1	2000	5000.0	0	11	Low
cyromazine	225	0.30	1000	3333.3	0	186	1.2	1	3100	10333.3	0	5	Negligible
deltamethrin	10	0.01	1290	96750.0	0	0.000079	126582.3	8	2000	150000.0	0	40	Medium
diazinon	10	0.01	65	4875.0	0	0.09	111.1	4	540	40500.0	0	20	Medium
dicamba	288	0.384	1000	2604.2	0	100	2.9	1	2000	5208.3	0	5	Negligible
Diclobenil	8100	10.80	1000	92.6	2	11	736.4	4	2000	185.2	1	33	Medium
difenoconazole	146	0.19	610	3133.6	0	187	0.8	0	2010	10325.3	0	0	Negligible
diflubenzuron	100	0.13	500	3750.0	0	100	1.0	1	2000	15000.0	0	5	Negligible
diflufenican	320	0.43	500	1171.9	0	100	3.2	1	2000	4687.5	0	5	Negligible
dimethoate	400	0.53	99.5	186.6	1	0.15	2666.7	8	2000	3750.0	0	46	High
dimethomorph	180	0.24	31	129.2	1	32.4	5.6	1	2000	8333.3	0	11	Low
dinocap	192	0.26	120	468.8	1	6.5	29.5	2	2000	7812.5	0	16	Low
diquate	800	1.07	130	121.9	1	13	61.5	2	424	397.5	1	18	Medium
dithianon	375	0.50	578	1156.0	0	25.4	14.8	2	2000	4000.0	0	10	Low
diuron	1275	1.70	400	235.3	1	100	12.8	2	5000	2941.2	0	16	Low
dodine	900	1.20	547	455.8	1	0.2	4500.0	8	1500	1250.0	0	46	High
esfenvalerate	15	0.02	212.5	10625.0	0	0.06	250.0	4	2000	100000.0	0	20	Medium
ethoprophos	10000	13.33	39.6	3.0	4	5.56	1798.6	8	26	2.0	1	64	Very high
famoxadone	30	0.04	470	11750.0	0	25	1.2	1	2000	50000.0	0	5	Negligible
fenamidone	100	0.13	25	187.5	1	159.8	0.6	0	2000	15000.0	0	6	Negligible
fenarimol	36	0.05	250	5208.3	0	10	3.6	1	2000	41666.7	0	5	Negligible
fenbuconazole	75	0.10	100	1000.0	1	5.2	14.4	2	5000	50000.0	0	16	Low
fenhexamid	750	1.00	1000	1000.0	1	100	7.5	1	5000	5000.0	0	11	Low
fenoxycarb	150	0.20	425	2125.0	0	0.1	1500.0	8	2000	10000.0	0	40	Medium
fluazifop-P-butyl	250	0.33	1000	3000.0	0	200	1.3	1	2000	6000.0	0	5	Negligible
fluazinam	200	0.27	1000	3750.0	0	100	2.0	1	2000	7500.0	0	5	Negligible
fludioxonil	200	0.27	1000	3750.0	0	329	0.6	0	2000	7500.0	0	0	Negligible
flufenacet	600	0.80	219	273.8	1	170	3.5	1	2000	2500.0	0	11	Low
flufenoxuron	100	0.13	1000	7500.0	0	109.1	0.9	0	2000	15000.0	0	0	Negligible
fluquinconazole	75	0.10	1000	10000.0	0	100	0.8	0	625	6250.0	0	0	Negligible
flusilazole	40	0.05	388	7275.0	0	150	0.3	0	2000	37500.0	0	0	Negligible
folpet	1250	1.67	1000	600.0	1	236	5.3	1	4500	2700.0	0	11	Low
foramsulfuron	56.25	0.08	453	6040.0	0	226	0.2	0	2000	26666.7	0	0	Negligible
fosetyl-aluminium	2000	2.67	1000	375.0	1	461.8	4.3	1	2000	750.0	1	13	Low
glufosinate-ammonium	1500	2.00	1000	500.0	1	100	15.0	2	2000	1000.0		16	Low
glyphosate	900	1.20	480	400.0	1	100	9.0	1	5000	4166.7	0	11	Low
imazalil	375	0.50	541	1082.0	0	40	9.4	1	4200	8400.0	0	5	Negligible

***Table K.1.2**. Results from the PRIHS-1 index.*

			EARTHORMS			BENEFICIAL ARTHROPODS			MAMMALS				
Pesticides	MRA (g/ha)	PEC=MRA/750	LC_{50}	LC_{50}/PEC	SCORE (A)	LD_{50}	%Efeito (MRA/LD_{50})	SCORE (B)	LD_{50cut}	LD_{50der}/PEC	SCORE (C)	PRIHS-1	Final score
imidacloprid	103	0.14	10.7	77.9	2	0.0037	27837.8	8	5000	36407.8	0	51	High
indoxacarb	37.5	0.05	1250	25000.0	0	23.33	1.6	1	5000	100000.0	0	5	Negligible
iprovalicarb	126	0.17	1000	5952.4	0	199	0.6	0	5000	29761.9	0	0	Negligible
isoxaben	1000	1.33	500	375.0	1	100	10.0	2	2000	1500.0	0	16	Low
lambda-cyhalothrin	20	0.03	1000	37500.0	0	0.038	526.3	4	632	23700.0	0	20	Medium
linuron	1000	1.33	1000	750.0	1	160	6.3	1	2000	1500.0	0	11	Low
lufenuron	100	0.13	1000	7500.0	0	197	0.5	0	2000	15000.0	0	0	Negligible
malathion	3040	4.05	613	151.2	1	0.16	19000.0	4	2000	493.4	1	28	Medium
mancozebe	1650	2.20	1000	454.5	1	209	7.9	1	5000	2272.7	0	11	Low
mesotrione	150	0.20	437.7	2188.5	0	11	13.6	1	2000	10000.0	0	5	Negligible
metalaxil-M	100	0.13	830	6225.0	0	127	0.8	0	2000	15000.0	0	0	Negligible
methidation	630	0.84	5.6	6.7	4	0.13	4846.2	8	200	238.1	1	64	Very High
methiocarb	150	0.20	200	1000.0	1	0.23	652.2	4	2000	10000.0	0	26	Medium
methomyl	380	0.51	21	41.4	2	0.28	1357.1	8	2000	3947.4	0	51	High
methoxyfenozide	960	1.28	1213	947.7	1	100	9.6	1	5000	3906.3	0	11	Low
metiram	1600	2.13	1000	468.8	1	80	20.0	2	2000	937.5	1	18	Medium
metribuzin	840	1.12	331.8	296.3	1	35	24.0	2	20000	17857.1	0	16	Low
myclobutanil	45	0.06	99	1650.0	0	171	0.3	0	5000	83333.3	0	0	Negligible
oxamyl	2	0.00	112	42000.0	0	0.38	5.3	1	2000	750000.0	0	5	Negligible
oxifluorfen	960	1.28	1000	781.3	1	100	9.6	1	10000	7812.5	0	11	Low
oxydemeton-methyl	500	0.67	115	172.5	1	0.31	1612.9	8	130	195.0	1	48	High
paraquat dichloride	1100	1.47	1380	940.9	1	36	30.6	2	200	136.4	1	18	Medium
penconazole	35	0.05	1000	21428.6	0	5	7.0	1	3000	64285.7	0	5	Negligible
phosalone	600	0.80	22.5	28.1	2	4.5	133.3	4	1500	1875.0	0	31	Medium
phosmet	300	0.40	52	130.0	1	1	300.0	4	5000	12500.0	0	26	Medium
pirimicarb	375	0.50	60	120.0	1	4	93.8	2	500	1000.0	1	18	Medium
procymidone	700	0.93	1000	1071.4	0	100	7.0	1	2500	2678.6	0	5	Negligible
propamocarb hydrochloride	722	0.96	660	685.6	1	84	8.6	1	3000	3116.3	0	11	Low
propiconazole	50	0.07	686	10290.0	0	100	0.5	0	4000	60000.0	0	0	Negligible
propineb	1750	2.33	700	300.0	1	70	25.0	2	5000	2142.9	0	16	Low
pymetrozine	300	0.40	250	625.0	1	117	2.6	1	2000	5000.0	0	11	Low
pyrimethanil	300	0.40	625	1562.5	0	100	3.0	1	5000	12500.0	0	5	Negligible
quinoxyfen	50	0.07	923	13845.0	0	100	0.5	0	2000	30000.0	0	0	Negligible
quizalofop-P -ethyl	150	0.20	1000	5000.0	0	100	1.5	0	5000	25000.0	0	0	Negligible
rimsulfuron	15	0.02	1000	50000.0	0	100	0.2	0	2000	100000.0	0	0	Negligible
s- metolachlor	1500	2.00	570	285.0	1	85	17.6	2	2000	1000.0	1	18	Medium
spinosad	120	0.16	1000	6250.0	0	0.0029	41379.3	8	2000	12500.0	0	40	Medium
spirodiclofen	960	1.28	1000	781.3	1	196	4.9	1	2000	1562.5	1	13	Low
sulcotrione	600	0.80	1000	1250.0	2	50	12.0	2	4000	5000.0	0	21	Medium
tau-fluvalinate	144	0.19	500	2604.2	1	5.83	24.7	2	2000	10416.7	0	16	Low
tebuconazole	100	0.13	1381	10357.5	0	83	1.2	1	5000	37500.0	0	5	Negligible
tebufenozide	144	0.19	1000	5208.3	0	234	0.6	0	2000	10416.7	0	0	Negligible
teflubenzuron	52.5	0.07	1000	14285.7	0	72	0.7	0	2000	28571.4	0	0	Negligible
terbutilazine	2415	3.22	200	62.1	2	100	24.2	2	2000	621.1	1	23	Medium
tetraconazole	50	0.07	1000	15000.0	0	130	0.4	0	2000	30000.0	0	0	Negligible
thiacloprid	96	0.13	105	820.3	1	17.32	5.5	1	2000	15625.0	0	11	Low
thiamethoxam	75	0.10	1000	10000.0	0	0.24	312.5	4	2000	20000.0	0	20	Medium
thiram	1600	2.13	540	253.1	1	2000	0.8	0	2000	937.5	1	8	Low
tolylfluanid	1000	1.33	1000	750.0	1	197	5.1	1	5000	3750.0	0	11	Low
trifloxystrobin	75	0.10	1000	10000.0	0	200	0.4	0	2000	20000.0	0	0	Negligible
trifluralin	1200	1.60	1000	625.0	1	100	12.0	2	5000	3125.0	0	16	Low
ziram	1800	2.40	190	79.2	2	100	18.0	2	2000	833.3	1	23	Medium
zoxamide	149.94	0.20	1070	5352.1	0	100	1.5	1	2000	10004.0	0	5	Negligible

EC_{50} - Median effective concentration; LD_{50der} - median lethal dose (by dermal contact);

MRA - Maximum application rate; PEC - Predicted environmental concentration

***Table K.2**. Results from the PRIHS-2 index.*

									(A) Earthorms		(B)Microrganisms	(C)Beneficial arthropods	(D) Mammals					
Pesticides	PEC	DT50	K	PEC earthorms (t=14)	PECmammals(T=730)	logkow	LC_{50}	LC_{50}/PEC	Score		Score	Score	BCF*	CD=(BCF*PECit)	NOEL	NOEL/CD	Score	PRIHS-2	Final score
abamectin	0.02	28	0.02	0.02	0.00	4.4	28	1840.7	0		0.1	8	69	1.2	4.5	3.623	4	30	High
acrinathrin	0.10	100	0.01	0.10	0.02	5.6	1000	10493.0	0		0.1	0	5902	590.2	2.4	0.004	8	12	Low
alpha-cypermethrin	0.04	91	0.01	0.04	0.01	6.94	100	2635.7	0		0.1	4	1204	48.2	1.5	0.031	8	24	Medium
benalaxyl	0.21	77	0.01	0.20	0.03	3.54	180	898.0	1		8	1	57	12.2	100	8.224	4	45	High
bentazone	1.60	12	0.06	1.10	0.04	-0.46	870	792.9	1		0.1	2	21	33.6	10	0.298	8	22	Medium
beta-cyfluthrin	0.02	13	0.05	0.01	0.00	5.9	1000	85154.5	0		0.1	2	506	8.4	60	7.115	4	12	Low
bitertanol	0.25	23	0.03	0.20	0.01	4.1	1000	4903.0	0		8	1	203	50.8	25	0.493	8	47	High
bupirimate	0.33	90	0.01	0.32	0.06	3.9	1000	3164.6	0		8	1	185	61.7	15	0.243	8	47	High
buprofezin	0.17	104	0.01	0.16	0.03	4.8	500	3142.1	0		0.1	0	509	84.8	0.9	0.011	8	12	Low
captan	2.67	1	0.69	0.27	0.01	2.8	519	1888.8	0		8	2	140	373.3	2000	5.357	4	44	High
carbaryl	1.33	28	0.02	1.13	0.07	1.85	106	94.1	2		0.1	4	44	58.7	200	3.409	4	26	Medium
carbendazime	0.80	32	0.02	0.69	0.05	1.51	6	8.7	4		8	2	25	20.0	300	15.000	2	57	Very high
cyazofamid	0.11	5	0.14	0.05	0.00	3.2	1000	21245.7	0		8	0	286	30.5	17	0.557	8	44	High
cyfluthrin	1.67	30	0.02	1.43	0.10	6	1000	700.9	1		0.1	8	506	845.0	50	0.059	8	40	High
cyprodinil	0.40	45	0.02	0.36	0.04	4	192	533.6	1		8	1	393	157.2	3	0.019	8	51	Very high
chlorpyrifos	1.28	56	0.01	1.18	0.14	4.2	210	178.7	1		0.1	8	1374	1758.7	0.1	0.000	8	40	High
chlorpyrifos-methyl	0.27	33	0.02	0.23	0.02	4.24	182	787.8	1		0.1	4	1800	480.0	0.1	0.000	8	28	Medium
deltamethrin	0.01	23	0.03	0.01	0.00	4.6	1290	118591.2	0		0.1	8	1400	18.7	1	0.054	8	36	High
diazinon	0.01	18	0.04	0.01	0.00	3.3	65	8364.0	0		0.1	4	500	5.0	0.015	0.003	8	24	Medium
dicamba	0.384	14	0.05	0.28	0.01	-1.88	1000	3610.1	0		0.1	1	15	5.8	110	19.097	2	6	Low
dicofol	0.80	30	0.02	0.68	0.05	4.3	43.1	63.1	2		0.1	2	10000	8000.0	0.22	0.000	8	26	Medium
difenoconazole	0.19	150	0.00	0.19	0.06	4.4	610	3236.0	0		8	0	320	62.3	1	0.016	8	44	High
diflubenzuron	0.13	7	0.10	0.07	0.00	3.89	500	6931.5	0		0.1	1	320	42.7	40	0.938	8	15	Medium
diflufenican	0.43	282	0.00	0.42	0.20	4.9	500	1192.2	0		0.1	1	1276	544.4	1000	1.837	4	9	Low
dinocap	0.26	24	0.03	0.21	0.01	4.54	120	569.9	1		8	2	992	254.0	0.4	0.002	8	54	Very high
diquate	1.07	365	0.00	1.06	0.58	-4.6	130	123.1	1		0.1	2	1	1.1	8.9	8.318	8	22	Medium
dithianon	0.50	35	0.02	0.44	0.03	3.2	578	1323.7	0		8	2	27	13.5	2.8	0.207	8	50	High
esfenvalerate	0.02	287	0.00	0.02	0.01	6.22	212.5	10805.6	0		0.1	4	3250	65.0	2	0.031	8	24	Medium
ethoprophos	13.33	7	0.10	7.21	0.18	3.59	39.6	5.5	4		0.1	8	225	3000.0	100	0.033	8	52	Very high
famoxadone	0.04	28	0.02	0.03	0.00	4.65	470	13903.5	0		8	1	3000	120.0	1.2	0.010	8	47	High
fenazaquine	66.67	45	0.02	59.97	5.93	5.51	26.5	0.4	8		0.1	8	500	33333.3	0.5	0.000	8	68	Very high
fenbuconazole	0.10	306	0.00	0.10	0.05	3.23	100	1015.9	0		8	2	160	16.0	6.4	0.400	8	50	High
fluazifop-P-butyl	0.33	28	0.02	0.28	0.02	4.95	1000	3549.8	0		0.1	1	320	106.7	1	0.009	8	15	Medium
fluazinam	0.27	27	0.03	0.22	0.01	4.1	1000	4478.4	0		8	1	1025	273.3	3.48	0.013	8	47	High
fludioxonil	0.27	25	0.03	0.22	0.01	4.12	1000	4524.8	0		8	0	366	97.6	40	0.410	8	44	High
flufenacet	0.80	32	0.02	0.69	0.05	3.2	1000	1449.1	0		0.1	1	71.4	57.1	1.67	0.029	8	15	Medium
fluquinconazole	0.10	300	0.00	0.10	0.05	3.24	1000	10162.6	0		8	0	87	8.7	0.31	0.036	8	44	High
flusilazole	0.05	95	0.01	0.05	0.01	3.74	388	7652.9	0		8	0	250	13.3	10	0.750	8	44	High
folpet	1.67	4	0.16	0.66	0.01	3.11	1000	1512.4	0		8	1	56	93.3	44.5	0.477	8	47	High
phosalone	0.80	4	0.17	0.30	0.01	4.01	22.5	74.8	2		0.1	4	180	144.0	2.5	0.017	8	32	High
glyphosate	1.20	130	0.01	1.16	0.30	-3.7	480	415.1	1		0.1	1	0.5	0.6	410			7	Low
imidacloprid	0.14	0	4.16	0.00	0.00	0.57	10.7	4536.4	0		0.1	8	0.61	0.1	100	1193.697	0	24	Medium
indoxacarb	0.05	186	0.00	0.05	0.02	4.65	1250	25657.8	0		0.1	1	520	26.0	40	1.538	4	9	Low
iprovalicarb	0.17	17	0.04	0.13	0.01	3.18	1000	7720.1	0		8	0	10	1.7	196	115.294	0	32	High
isoxaben	1.33	120	0.01	1.28	0.31	3.94	500	391.3	1		0.1	2	70.5	93.8	5.6	0.060	8	22	Medium
lambda-cyhalothrin	0.03	40	0.02	0.02	0.00	7	1000	42232.5	0		0.1	4	1950	52.0	0.5	0.010	8	24	Medium
lufenuron	0.13	20	0.03	0.11	0.01	5.12	1000	9466.1	0		0.1	0	5300	706.7	2	0.003	8	12	Low
malathion	4.05	0	3.85	0.08	0.00	2.75	613	8159.9	0		0.1	4	103	417.2	500	1.199	4	18	Medium
mancozebe	2.20	1	0.69	0.23	0.00	0.26	1000	4411.2	0		8	1	3.2	7.0	4.8	0.682	8	47	High
metalaxil-M	0.13	21	0.03	0.11	0.01	1.71	830	7773.7	0		8	0	15	2.0	250	125.000	1	34	High
methidation	0.84	18	0.04	0.65	0.03	2.2	5.6	8.6	4		0.1	8	75	63.0	4	0.063	8	52	Very high
metiram	2.13	6	0.12	1.06	0.03	0.3	1000	947.3	1		8	2	3.2	6.8	3.1	0.455	8	54	Very high
methiocarb	0.20	35	0.02	0.17	0.01	3.08	200	1145.0	0		0.1	4	75	15.0	60	4.000	4	18	Medium
methoxyfenozide	1.28	268	0.00	1.26	0.58	3.7	1213	964.9	1		0.1	1	11	14.1	10	0.710	8	19	Medium
metribuzin	1.12	60	0.01	1.03	0.13	1.6	331.8	320.9	1		0.1	2	10	11.2	100	8.929	4	16	Medium
oxamyl	0.00	7	0.10	0.00	0.00	-0.44	112	77632.5	0		8	1	2	0.0	50	9375.000	0	35	High
oxifluorfen	1.28	55	0.01	1.17	0.14	4.47	1000	852.2	1		0.1	1	1637	2095.4	2	0.001	8	19	Medium
penconazole	0.05	343	0.00	0.05	0.02	3.72	1000	21733.1	0		8	1	320	14.9	0.71	0.048	8	47	High
pirimicarb	0.50	234	0.00	0.49	0.20	1.7	60	122.5	1		0.1	2	24	12.0	3.5	0.292	8	22	Medium
procymidone	0.93	84	0.01	0.88	0.15	3.14	1000	1134.5	0		8	1	46.95	43.8	300	6.846	4	41	High
propamocarb hydrochloride	0.96	30	0.02	0.82	0.06	-1.21	660	802.4	1		8	1	54	52.0	26	0.500	8	51	Very high
propiconazole	0.07	70	0.01	0.06	0.01	3.72	686	11019.7	0		8	0	116	7.7	3.6	0.466	8	44	High
propineb	2.33	3	0.23	0.69	0.01	-0.26	700	1011.6	0		8	2	821	1912.9	50	0.026		38	High
quinoxyfen	0.07	454	0.00	0.07	0.04	4.66	923	13993.5	0		8	0	5040	336.0	20	0.060	8	44	High
quizalofop-P -ethyl	0.20	1	0.76	0.02	0.00	4.61	1000	52932.6	0		0.1	0	380	76.0	1.3	0.017	8	12	Low
spinosad	0.16	1	1.39	0.01	0.00	4	1000	121300.8	0		0.1	8	0.1	0.0	9	562.500	0	24	Medium
spirodiclofen	1.28	7	0.09	0.71	0.02	5.8	1000	1412.3	0		0.1	1	1.4	1.8	6	3.348	2	6	Low
s- metolachlor	2.00	30	0.02	1.71	0.12	3.05	579	338.8	1		0.1	2	68.8	137.6	15	0.109	8	22	Medium
tau-fluvalinate	0.19	92	0.01	0.18	0.03	4.26	500	2772.8	0		0.1	2	200	38.0	1	0.026	8	18	Medium
tebuconazole	0.13	28	0.02	0.11	0.01	3.7	1381	12255.8	0		8	1	78	10.4	20	1.923	4	41	High
tebufenozide	0.19	348	0.00	0.19	0.10	4.25	1000	5281.3	0		0.1	0	70	13.4	5.5	0.409	8	12	Low
teflubenzuron	0.07	84	0.01	0.07	0.01	4.3	1000	15126.8	0		0.1	0	654	45.8	4.1	0.090	8	12	Low
thiram	2.13	210	0.00	2.08	0.81	1.73	540	259.0	1		8	0	6	12.8	1.5	0.117	8	48	High
tolylfluanid	1.33	2	0.35	0.27	0.01	3.9	1000	3667.7	0		8	1	74	98.7	12	0.122	8	47	High
trifluralin	1.60	126	0.01	1.54	0.39	4.83	1000	649.4	0		0.1	2	5674	9078.4	813	0.090	8	18	Medium
zoxamide	0.20	10	0.07	0.13	0.00	3.76	1070	8362.6	0		8	1	115	23.0	50	2.175	2	38	High

EC_{50} - Median effective concentration; PEC - Predicted environmental concentration

MRA - Maximum application rate; NOEL - No-observed-effect-level; BCF - Bioconcentration factor

***Table K.3**. Results from the PRIES-1 index.*

			Bees			Birds			Beneficial arthropods	Mammals				
Pesticides	MRA	ADI	LD_{50}	HQ=MRA/LD 50	Score	LD_{50}	LD_{50}/ADI	Score	Score	LD_{50}(oral)	LD50/ADI	Score	PRIES-1	Final score
abamectin	13.5	0.002	0.0022	6136.36	8	84.6	42300	0	8	10	5000	0	48	Medium
acetamiprid	100	0.07	14.5	6.90	1	98	1400	0	1	213	3042.85714	0	6	Low
acrinathrin	75	0.02	175	0.43	0	1000	50000	0	0	5000	250000	0	0	Negligible
alpha-cypermethrin	30	0.02	0.059	508.47	4	2025	101250	0	4	57	2850	0	24	Medium
amitrole	3600	0.001	150	24.00	2	2150	2150000	0	2	5000	5000000	0	12	Low
azoxystrobin	200	0.1	25	8.00	1	2000	20000	0	1	5000	50000	0	6	Low
benalaxyl	160	0.05	100	1.60	1	4500	90000	0	1	680	13600	0	6	Low
bentazone	1200	0.1	100	12.00	2	1140	11400	0	2	500	5000	0	12	Low
bifenthrin	80	0.02	0.1	800.00	4	1800	90000	0	4	54.5	2725	0	24	Medium
bitertanol	187.5	0.01	104.4	1.80	1	776	77600	0	1	4300	430000	0	6	Low
bromoxinil	450	0.01	5	90.00	2	217	21700	0	2	1000	100000	0	12	Low
buprofezin	125	0.01	163.5	0.76	0	2000	200000	0	0	2198	219800	0	0	Negligible
captan	2000	0.1	100	20.00	2	2000	20000	0	2	9000	90000	0	12	Low
carbaryl	1000	0.008	1	1000.00	4	1000	125000	0	4	264	33000	0	24	Medium
carbofuran	500	0.002	0.04	12500.00	8	2.5	1250	0	8	8	4000	0	48	Medium
chlorothalonil	1500	0.03	63	23.81	2	4640	154666.667	0	2	5000	166666.667	0	12	Low
chlorpyrifos	960	0.01	0.36	2666.67	8	32	3200	0	8	135	13500	0	48	Medium
chlorpyrifos-methyl	200	0.01	0.38	526.32	4	932	93200	0	4	1100	110000	0	24	Medium
cycloxydim	400	0.07	100	4.00	1	2000	28571.4286	0	1	5000	71428.5714	0	6	Low
cyazofamid	80	0.17	151.7	0.53	0	5000	29411.7647	0	0	5000	29411.7647	0	0	Negligible
cyfluthrin	1250	0.02	0.001	1250000	8	2000	100000	0	8	20	1000	1	50.5	High
cymoxanil	120	0.013	25	4.80	1	2250	173076.923	0	1	760	58461.5385	0	6	Low
cypermethrin	100	0.05	0.035	2857.14	8	2000	40000	0	8	138	2760	0	48	Medium
cyprodinil	300	0.03	100	3.00	1	500	16666.6667	0	1	2000	66666.6667	0	6	Low
cyromazine	225	0.02	186	1.21	1	1000	50000	0	1	3387	169350	0	6	Low
deltamethrin	10	0.01	0.000079	126582.28	8	4640	464000	0	8	300	30000	0	48	Medium
diazinon	10	0.002	0.09	111.11	4	2.7	1350	0	4	80	40000	0	24	Medium
difenoconazole	146	0.01	187	0.78	0	2150	215000	0	0	1453	145300	0	0	Negligible
diflubenzuron	100	0.02	100	1.00	1	2000	100000	0	1	4640	232000	0	6	Low
diflufenican	320	0.185	100	3.20	1	2150	11621.6216	0	1	2150	11621.6216	0	6	Low
dimethoate	400	0.002	0.15	2666.67	8	14.1	7050	0	8	160	80000	0	48	Medium
dimethomorph	180	0.05	32.4	5.56	1	2000	40000	0	1	3900	78000	0	6	Low
dinocap	192	0.008	6.5	29.54	2	2150	268750	0	2	990	123750	0	12	Low
diquate	800	0.002	13	61.54	2	83	41500	0	2	218	109000	0	12	Low
diuron	1275	0.002	100	12.75	2	1104	552000	0	2	437	218500	0	12	Low
dodine	900	0.2	0.2	4500.00	8	788	3940	0	8	1000	5000	0	48	Medium
esfenvalerate	15	0.02	0.06	250.00	4	381	19050	0	4	75	3750	0	24	Medium
ethoprophos	10000	0.0004	5.56	1798.56	8	5.6	14000	0	8	55	137500	0	48	Medium
famoxadone	30	0.006	25	1.20	1	2250	375000	0	1	5000	833333.333	0	6	Low
fenamidone	100	0.04	159.8	0.63	0	2000	50000	0	0	2028	50700	0	0	Negligible
fenamiphos	3400	0.0008	0.28	12142.86	8	0.7	875	1	8	6	7500	0	52	High
fenbuconazole	75	0.006	5.2	14.42	2	2110	351666.667	0	2	2000	333333.333	0	12	Low
fenhexamid	750	0.183	100	7.50	1	2000	10928.9617	0	1	5000	27322.4044	0	6	Low
fluazifop-P-butyl	250	0.01	200	1.25	1	3500	350000	0	1	2451	245100	0	6	Low
fluazinam	200	0.01	100	2.00	1	1782	178200	0	1	5000	500000	0	6	Low
fludioxonil	200	0.4	329	0.61	0	2000	5000	0	0	5000	12500	0	0	Negligible
flufenacet	600	0.005	170	3.53	1	1608	321600	0	1	1.67	334	1	8.5	Low
flufenoxuron	100	0.035	109.1	0.92	0	2000	57142.8571	0	0	3000	85714.2857		0	Negligible
flusilazole	40	0.001	150	0.27	0	1590	1590000	0	0	674	674000	0	0	Negligible
folpet	1250	0.1	236	5.30	1	2000	20000	0	1	9000	90000	0	6	Low
fosetyl-aluminium	2000	2.98	461.8	4.33	1	8000	2684.56376	0	1	7080	2375.83893	0	6	Low
glufosinate-ammonium	1500	0.02	100	15.00	2	2000	100000	0	2	416	20800	0	12	Low
glyphosate	900	0.3	100	9.00	1	3851	12836.6667	0	1	3530	11766.6667	0	6	Low
imidacloprid	103	0.06	0.0037	27837.84	8	31	516.666667	1	8	450	7500	0	52	High
iprovalicarb	126	0.015	199	0.63	0	2000	133333.333	0	0	5000	333333.333	0	0	Negligible
isoxaben	1000	0.056	100	10.00	1	2000	35714.2857	0	1	5000	89285.7143	0	6	Low
lambda-cyhalothrin	20	0.005	0.038	526.32	4	3950	790000	0	4	56	11200	0	24	Medium
lufenuron	100	0.01	197	0.51	0	2000	200000	0	0	2000	200000	0	0	Negligible
malathion	3040	0.03	0.13	23384.62	8	359	11966.6667	0	4	775	25833.3333	0	36	Medium
mancozebe	1650	0.03	209	7.89	1	1290	43000	0	1	5000	166666.667	0	6	Low
mesotrione	150	0.01	11	13.64	2	2000	200000	0	1	5000	500000	0	9	Low
metalaxil-M	100	0.08	127	0.79	0	981	12262.5	0	0	375	4687.5	0	0	Negligible
methidation	630	0.001	0.13	4846.15	8	23.6	23600	0	8	25	25000	0	48	Medium
methiocarb	150	0.02	0.23	652.17	4	7.1	355	1	4	25	1250	0	28	Medium
methomyl	380	0.02	0.28	1357.14	8	24.2	1210	0	8	30	1500	0	48	Medium
metiram	1600	0.03	80	20.00	2	2150	71666.6667	0	2	5000	166666.667	0	12	Low
metribuzin	840	0.013	35	24.00	2	164	12615.3846	0	2	250	19230.7692	0	12	Low
myclobutanil	45	0.03	171	0.26	0	510	17000	0	0	1600	53333.3333	0	0	Negligible
oxamyl	2	782	0.38	5.26	1	3.16	0.00404092	8	1	2.5	0.00319693	8	58	High
oxifluorfen	960	0.003	100	9.60	1	2150	716666.667	0	1	5000	1666666.67	0	6	Low
paraquat dichloride	1100	0.004	36	30.56	2	175	43750	0	2	30	7500	0	12	Low
penconazole	35	0.03	5	7.00	1	1590	53000	0	1	2125	70833.3333	0	6	Low
phosalone	600	0.02	4.5	133.33	4	2150	107500	0	4	120	6000	0	24	Medium
phosmet	300	0.01	1	300.00	4	57	5700	0	4	113	11300	0	24	Medium
procymidone	700	0.1	100	7.00	1	4092	40920	0	1	6800	68000	0	6	Low
propamocarb hydrochloride	722	0.4	84	8.60	1	1842	4605	0	1	1450	3625	0	6	Low
propiconazole	50	0.02	100	0.50	0	2223	111150	0	0	1490	74500	0	0	Negligible
propineb	1750	0.0003	70	25.00	2	5000	16666666.7	0	2	2500	8333333.33	0	12	Low
pymetrozine	300	0.03	117	2.56	1	2000	66666.6667	0	1	5820	194000	0	6	Low
pyrimethanil	300	0.02	100	3.00	1	2000	100000	0	1	4150	207500	0	6	Low
quinoxyfen	50	0.2	100	0.50	0	2250	11250	0	0	5000	25000	0	0	Negligible
s- metolachlor	1500	0.1	85	17.65	2	2510	25100	0	2	2000	20000	0	12	Low
spinosad	120	0.024	0.0029	41379.31	8	2000	83333.3333	0	8	3783	157625	0	48	Medium
spirodiclofen	960	0.015	196	4.90	1	2000	133333.333	0	1	2500	166666.667	0	6	Low
sulcotrione	600	0.005	50	12.00	2	1350	270000	0	2	5000	1000000	0	12	Low
tau-fluvalinate	144	0.01	5.83	24.70	2	2510	251000	0	2	261	26100	0	12	Low
tebuconazole	100	0.03	83	1.20	1	1988	66266.6667	0	1	1700	56666.6667	0	6	Low
tebufenozide	144	0.02	234	0.62	0	2150	107500	0	0	5000	250000	0	0	Negligible
terbutilazine	2415	0.0022	100	24.15	2	1000	454545.455	0	2	1590	722727.273	0	12	Low
thiacloprid	96	0.12	17.32	5.54	1	49	408.333333	1	1	396	3300	0	10	Low
thiamethoxam	75	0.026	0.24	312.50	4	576	22153.8462	0	4	1563	60115.3846	0	24	Medium
tolylfluanid	1000	0.08	197	5.08	1	2000	25000	0	1	5000	62500	0	6	Low
trichlorfon	1200	0.01	0.4	3000.00	8	36.8	3680	0	8	250	25000	0	48	Medium
trifluralin	1200	0.024	100	12.00	2	2000	83333.3333	0	2	5000	208333.333	0	12	Low
ziram	1800	0.003	100	18.00	2	97	32333.3333	0	2	100	33333.3333	0	12	Low
zoxamide	149.94	0.5	100	1.50	1	2000	4000	0	1	5000	10000	0	6	Low

HQ - Hazard coefficient; LD_{50}- Median lethal dose;
MRA - Maximum application rate; ADI - Acceptable daily intake

***Table K.4**. Results from the PRIES-2 index.*

	Persistence		Bioaccumulation		Air affinity		Soil affinity		Application rate		Plants	Bees		Beneficial arthropods	Birds		Mammals			
Pesticides	DT_{50} (d)	Score	logKOW	Score	%	Score	%	Score	MRA(g/ha)	Score	Score	LD_{50}	Score	Score	LD_{50}	Score	NOEL	Score	PRIES-2	Final score
abamectin	28	2	4.4	1.25	3.95E-01	1.25	92.9	1.5	13.5	1	0.1	0.0022	4	8	84.6	1	4.5	2	10	Low
acetamiprid	5	1	0.8	1	1.07E-06	1	0.556	1	100	2	0.1	14.5	1	1	98	1	15	1	2	Negligible
acrinathrin	100	4	5.6	1.25	3.77E-04	1	10.5	1.25	75	2	0.1	175	0.1	0	1000	0.1	2.4	2	5	Low
alpha-cypermethrin	91	4	6.94	1.25	6.27E-03	1	97.7	1.5	30	1	0.1	0.059	4	4	2025	0.1	1.5	2	13	Low
azoxystrobin	14	2	2.5	1.1	6.91E-08	1	49.8	1.5	200	2	0.1	25	1	1	2000	0.1	18	1	4	Negligible
benalaxyl	77	3	3.54	1.25	3.72E-02	1.25	74.1	1.5	160	2	0.1	100	1	1	4500	0.1	100	1	7	Low
benalaxyl-M	124	4	3.67	1.25	3.89E-07	1	0.344	1	12.5	1	0.1	0.001							0	Negligible
benoxacor	5	1	2.6	1.1	1.16E-01	1.25	25.9	1.5	75	2	4	100	1	0	2000	0.1	0.5	3	5	Negligible
bentazone	12	2	-0.46	1	4.67E-05	1	0.0307	1	1200	4	4	100	1	2	1140	0.1	10	2	15	Low
beta-cyfluthrin	13	2	5.9	1.25	3.89E-07	1	0.344	1	12.5	1	0.1	0.001			2000		60	1	1	Negligible
bifenthrin	125	4	6	1.25	2.28E-01	1.25	97.1	1.5	80	2	0.1	0.1		4	1800	0.1	1.5	2	17	Medium
bromoxinil	1	1	1.04	1	1.07E-05	1	0.961	1	450	3	0.1	5	2	2	217	2	20	1	4	Negligible
bupirimate	90	3	3.9	1.25	6.06E-03	1	85.8	1.5	250	3	0.1	50	1	1	2700	0.1	15	1	9	Low
buprofezin	104	4	4.8	1.25	1.17E-02	1.25	96.1	1.5	125	2	0.1	163.5	0.1	0	2000	0.1	0.9	3	9	Low
captan	1	1	2.8	1.1	1.52E+00	1.25	35	1.5	2000	4	0.1	100	1	2	2000	0.1	2000	0.1	4	Negligible
carbaryl	28	2	1.85	1	1.33E-03	1	5.89	1.25	1000	3	0.1	1	3	4	1000	0.1	200	0.1	10	Low
carbendazime	32	3	1.51	1	7.14E-02	1.25	2.78	1.25	600	3	0.1	50	1	2	5826	0.1	300	0.1	7	Low
carbofuran	60	3	1.52	1	9.92E-04	1	2.85	1.25	500	3	0.1	0.04	4	8	2.5	2	10	2	33	Medium
chlorothalonil	28	2	2.92	1.1	2.91E-01	1.25	41.9	1.5	1500	5	0.1	63	1		4640	0.1	3	2	10	Low
chlorpyrifos-methyl	33	3	4.24	1.25	4.54E-01	1.25	91.5	1.5	200	2	0.1	0.38	3	4	932	0.1	0.1	3	21	Medium
clofentezine	132	4	4.1	1.25	2.79E-02	1.25	89.4	1.5	duvida		0.1	252.6	0.1		3000	0.1	40	1	0	Negligible
cyazofamid	5	1	3.2	1.1	3.31E-01	1.25	57.4	1.5	80	2	0.1	151.7	0.1	0	0.1	0.1	17	1	1	Negligible
cycloxydim	1	1	1.36	1	1.23E-03	1	1.99	1.25	400	3	4	100	1	1	2000	0.1	7	2	5	Low
cymoxanil	9	1	0.59	1	7.79E-04	1	0.343	1	120	2	0.1	25	1	1	2250	0.1	3	2	2	Negligible
cypermethrin	60	3	6.6	1.25	1.18E-04	1	97.7	1.5	100	2	0.1	0.035	4		2000	0.1	0.05	4	15	Medium
cyprodinil	45	3	4	1.25	1.80E-02	1.25	88	1.5	300	3	0.1	100	1	1	500	0.1	3	2	13	Low
cyromazine	10	1	-0.061	1	1.17E-06	1	0.0769	1	225	3	0.1	**186**	0.1	1	1000	0.1	300	0.1	1	Negligible
deltamethrin	23	2	4.6	1.25	1.87E-04	1	0.238	1	10	1	0.1	0.079	4	8	4640	0.1	1	3	8	Low
dicamba	14	2	-1.88	1	3.03E-04	1	0.00117	1	288	2	4	100	1	1	2000	0.1	110	1	6	Low
dicofol	30	2	4.3	1.25	2.64E-02	1.25	92.6	1.5	600	3	0.1	50	1		1418	0.1	0.22	3	9	Low
diflubenzuron	7	1	3.89	1.25	1.19E-03	1	85.6	1.5	100	2	0.1	100	1	1	2000	0.1	40	1	2	Negligible
diflufenican	282	4	4.9	1.25	9.42E-03	1	96.4	1.5	320	3	4	100	1	1	2150	0.1	1000	0.1	23	Medium
dimethoate	16	2	0.704	1	5.02E-04	1	0.446	1	400	3	0.1	0.15	3	8	14.1	1	0.2	3	18	Medium
dimethomorph	53	3	2.63	1.1	1.13E-04	1	27.2	1.5	180	2	0.1	32.4	1	1	2000	0.1	9	2	7	Low
dinocap	24	2	4.54	1.25	5.08E-03	1	94.6	1.5	192	2	0.1	6.5	2	2	2150	0.1	0.4	3	9	Low
dithianon	35	3	3.2	1.1	4.80E-05	1	57.6	1.5	375	3	0.1	25.4	1	2	290	0.1	2.8	2	13	Low
dodine	22	2	1.65	1	8.99E-05	1	3.8	1.25	900	3	0.1	0.2	3	8	788	0.1	800	0.1	15	Medium
esfenvalerate	287	4	6.22	1.25	5.72E-04	1	97.6	1.5	15	1	0.1	0.06	4	4	381	0.1	2	2	13	Low
famoxadone	28	2	4.65	1.25	2.28E-03	1	95.4	1.5	30	1	0.1	25	1	1	2250	0.1	1.2	2	3	Negligible
fenamidone	97	4	2.8	1.1	1.77E-04	1	35.6	1.5	100	2	0.1	159.8	0.1	0	2000	0.1	3.6	2	5	Low
fenazaquine	45	3	5.51	1.25	3.30E-04	1	97.4	1.5	50000	5	0.1	1.21	2		1747	0.1	0.5	3	24	Medium
fenoxycarb	31	3	4.07	1.25	5.83E-05	1	89.4	1.5	150	2	0.1	0.1	4		7000	0.1	5.5	2	12	Low
fluazifop-P-butyl	28	2	4.95	1.25	2.28E-02	1	96.5	1.5	250	3	4	200	0.1	1	3500	0.1	1	3	15	Medium
fluazinam	27	2	4.1	1.25	2.99E+01	2	62.9	1.5	200	2	0.1	**100**	1	1	1782	0.1	**3.48**	2	7	Low
fludioxonil	25	2	4.12	1.25	9.99E+01	1.5	0.106	1	200	2	0.1	329	0.1	0	2000	0.1	40	1	2	Negligible
flufenacet	32	3	3.2	1.1	4.29E-03	1	57.6	1.5	600	3	4	170	0.1	1	1608	0.1	1.67	2	18	Medium
fluquinconazole	300	4	3.24	1.1	1.46E-05	1	64.4	1.5	75	2	0.1	100	1	0	2000	0.1	0.31	3	9	Low
flusilazole	95	4	3.74	1.25	7.82E-04	1	81.4	1.5	40	1	0.1	150	0.1	0	1590	0.1	10	2	3	Negligible
folpet	4	1	3.11	1.1	7.36E-02	1	52.6	1.5	1250	4	0.1	236	0.1	1	2000	0.1	44.5	1	3	Negligible
fosetyl-aluminium	1	1	-2.4	1	6.53E-09	1	3.53E-04	1	2000	4	0.1	461.8	0.1	1	8000	0.1	298	0.1	1	Negligible
glufosinate-ammonium	20	2	0.1	1	2.96E-07	1	1.11E-01	1	1500	4	4	100	1	2	2000	0.1	2	2	15	Low
imidacloprid	0	1	0.57	1	3.48E-09	1	0.328	1	103	2	0.1	**0.0037**	4	8	31	1	100	1	6	Low
indoxacarb	186	4	4.65	1.25	2.97E-05	1	95.4	1.5	37.5	1	0.1	23.33	1	1	98	1	40	1	5	Low
isoxaben	120	4	3.94	1.25	2.97E-04	1	86.8	1.5	1000	4	4	100	1	2	2000	0.1	5.6	2	46	High
lambda-cyhalothrin	40	3	7	1.25	4.07E-05	1	97.7	1.5	20	1	0.1	**0.038**	4	4	3950	0.1	0.5	3	11	Low
lufenuron	20	2	5.12	1.25	5.80E-03	1	96.9	1.5	100	2	0.1	197	0.1	0	2000	0.1	2	2	3	Negligible
malathion	0	1	2.75	1	1.64E-01	1.25	32.9	1.5	3040	5	0.1	0.13	3	4	359	0.1	500	0.1	10	Low
mancozebe	1	1	0.26	1	1.19E-02	1.25	1.61E-01	1	1650	4	0.1	209	0.1	1	1290	0.1	4,8	2	3	Negligible
mesotrione	17	2	-1	1	1.80E-05	1	0.0088	1	150	2	4	11	1	1	2000	0.1	0.24	3	7	Low
metalaxil-M	21	2	1.71	1	6.95E-04	1	4.34	1.25	100	2	0.1	127	0.1	0	981	0.1	250	0.1	0	Negligible
methidation	18	2	2.2	1	6.78E-03	1	12.3	1.25	630	3	0.1	0.13	3	8	23.6	1	4	2	19	Medium
methiocarb	35	3	3.08	1.1	1.23E-03	1	51	1.5	150	2	0.1	0.23	3	4	7.1	2	60	1	17	Medium
methomyl	8	1	0.093	1	4.13E-05	1	0.11	1	380	3	0.1	0.28	3	8	24.2	1	50	1	8	Low
methoxyfenozide	268	4	3.7	1.25	6.12E-04	1	80.1	1.5	960	3	0.1	100	1	1	2250	0.1	10	2	16	Medium
metribuzin	60	3	1.6	1	2.34E-04	1	3.4	1.25	840	3	4	35	1	2	164	0.1	100	1	16	Medium
oxamyl	**7**	1	-0.44	1	**8.19E-06**	1	**0.0321**	1	2	1	0.1	0.38	3	1	3.16	2	50	1	1	Negligible
oxifluorfen	55	3	4.47	1.25	6.15E-02	1.25	94.1	1.5	960	3	4	100	1	1	2150	0.1	2	2	25	Medium
paraquat dichloride	2800	5	-4.5	1	8.51E-08	1	8.86E-05	1	1100	5	4	36	2	2	175	2	1.7	2	60	High
penconazole	343	5	3.72	1.25	2.36E-03	1	80.8	1.5	35	1	0.1	5	2	1	1590	0.1	0.71	3	10	Low
phosalone	4	1	4.01	1.25	1.44E-02	1.25	88.2	1.5	600	3	0.1	4.5	2	4	2150	0.1	2.5	2	8	Low
phosmet	7	1	2.95	1.1	9.36E-03	1	43.7	1.5	300	3	0.1	1	3	4	**57**	1	40	1	8	Low
pirimicarb	234	4	1.7	1	6.24E-04	1	4.25	1.25	375	3	0.1	4	2	2	20.9	1	3.5	2	19	Medium
procymidone	84	3	3.14	1.1	5.70E+00	1.5	51.2	1.5	700	3	0.1	100	1	1	4092	0.1	300	0.1	7	Low
propamocarb hydrochloride	30	2	-1.21	1	3.50E-07	1	5.46E-03	1	722	3	0.1	84	1	1	1842	0.1	26	1	4	Negligible
propiconazole	70	3	3.72	1.25	3.29E-04	1	80.8	1.5	50	2	0.1	100	1	0	2223	0.1	3.6	2	6	Low
propineb	3	1	-0.26	1	7.09E-07	1	0.0487	1	1750	4	0.1	70	1	2	5000	0.1	50	1	3	Negligible
pymetrozine	69	3	-0.18	1	6.14E-05	1	0.0585	1	300	3	0.1	117	0.1	1	2000	0.1	3.7	2	6	Low
pyrimethanil	54	3	2.84	1.1	4.57E-02	1.25	37.6	1.5	300	3	0.1	100	1	1	2000	0.1	20	1	9	Low
quinoxyfen	380	5	4.66	1.25	3.80E-02	1.25	95.4	1.5	50	2	0.1	100	1	0	2250	0.1	20	1	8	Low
quizalofop-P -ethyl	1	1	4.61	1.25	3.64E-05	1	95.2	1.5	150	2	4	100	1	0	2000	0.1	1.3	2	4	Negligible
rimsulfuron	20	2	-1.47	1	1.33E-03	1	0.003	1	15	1	4	100	1	0	2000	0.1	3000	0.1	2	Negligible
s- metolachlor	30	2	3.02	1.25	2.23E-02	1	49.3	1.5	1500	4	4	85	1	2	2510	0.1	15	1	20	Medium
spinosad	1	1	4	1.25	1.97E-07	1	88	1.5	120	2	0.1	0.0029	4	8	2000	0.1	9	2	9	Low
spirodiclofen	7	1	5.8	1.25	8.84E-04	1	97.6	1.5	960	3	0.1	196	0.1	1	2000	0.1	6	2	3	Negligible
tau-fluvalinate	92	4	4.26	1.25	2.36E-05	1	46	1.5	144	2	0.1	5.83	2	2	2510	0.1	1	3	18	Medium
tebuconazole	28	2	3.7	1.25	5.38E-05	1	80.1	1.5	100	2	0.1	83	1	1	1988	0.1	20	1	4	Negligible
tebufenozide	348	5	4.25	1.25	7.94E-05	1	92	1.5	144	2	0.1	234	0.1	0	2150	0.1	5.5	2	7	Low
teflubenzuron	84	3	4.3	1.25	7.43E-03	1	92	1.5	52.5	2	0.1	72	1	0	2250	0.1	4.1	2	6	Low
terbutilazine	60	3	3.21	1.25	3.37E-02	1.25	58.1	1.5	2415	5	4	100	1	2	1000	0.1	15.4	2	47	High
thiacloprid	7	1	1.26	1	3.56E-09	1	56.3	1.5	96	2	0.1	17.32	1	1	49	1	1.23	2	3	Negligible
thiram	210	4	1.73	1	5.97E-01	1.25	4.51	1.25	1600	4	0.1	0.065	4	0	100	1	1.5	2	28	Medium
tolylfluanid	2	1	3.9	1.25	1.93E-02	1.25	85.8	1.5	1000	3	0.1	197	0.1	1	2000	0.1	12	1	2	Negligible
trichlorfon	18	2	0.43	1	9.22E-06	1	2.38E-01	1	1200	4	0.1	0.4	3		36.8	1	100	1	8	Low
trifluralin	126	4	4.83	1.25	3.39E+00	1.25	92.9	1.5	1200	4	4	100	1	2	2000	0.1	813	0.1	40	medium
zoxamide	10	2	3.76	1.25	1.63E-02	1.25	82	1.5	149.94	2	0.1	100	1	1	2000	0.1	50	1	4	Negligible

DT_{50}- Half-life; Log_{KOW} - logarithm of the partition coefficient octanol/water;

MRA - Maximum application rate; LD50- Median lethal dose; NOEL - No-observed-effect-level

***Table K.5**. Results from the PRIWS-1 index.*

			Algae (A)			*Daphnia* (B)			Fish (C)				
Pesticides	Water affinity	PEC_{ST}	EC_{50}	EC_{50}/PEC	Score	EC_{50}	EC_{50}/PEC	Score	LC_{50}	LC_{50}/PEC	Score	PRISW-1	Final score
abamectin	4.17	1.00E-04	1000	10000000	0	0.00034	3.4	6	0.0032	32	4	46	High
acetamiprid	99.4	1.00E-01	93	930	2	200	2000	1	100	1000	1	16	Medium
acrinathrin	0.0383	1.00E-05	0.82	82000	0	0.57	57000	0	5.66	566000	0	0	Negligible
alpha-cypermethrin	0.0127	1.00E-05	0.1	10000	1	0.0001	10	4	0.0028	280	2	30	Medium
amitrole	4.01	1.00E-03	2.3	2300	1	6.1	6100	0	1000	1000000	0	3	Negligible
azoxystrobin	46.1	1.00E-03	0.12	120	2	0.08	80	4	0.47	470	2	33	Medium
benalaxyl	24.1	1.00E-03	2.4	2400	1	0.59	590	2	3.75	3750	1	17	Medium
benoxacor	73.4	1.00E-02	0.63	63	4	4.8	480	2	2.4	240	2	31	Medium
bentazone	100	1.00E-01	47.3	473	2	125	1250	1	100	1000	1	16	Medium
beta-cyfluthrin	94.8	1.00E-02	10	1000	1		0			0		3	Negligible
bifenthrin	0.11	1.00E-05	50	5000000	0	0.00016	16	4	0.00015	15	4	38	Medium
buprofezin	1.72	1.00E-05	2.1	210000	0	0.42	42000	0	0.527	52700	0	0	Negligible
captan	62.7	1.00E-02	91	9100	1	7.00	700	2	0.034	3.4	6	44	High
carbaryl	94	1.00E-02	1.1	110	2	0.006	0.6	8	1.3	130	2	49	High
carbendazime	97.1	1.00E-01	419	4190	1	0.13	1.3	8	0.61	6.1	6	68	High
carbofuran	97.1	1.00E-01	6.5	65	4	0.0386	0.386	8	1.75	17.5	4	66	High
chlorothalonil	56.9	1.00E-03	0.21	210	2	0.07	70	4	0.047		4	44	High
chlorpyrifos	2.15	1.00E-04	0.4	4000	1	0.0017	17	4	0.0007	7	6	52	High
chlorpyrifos-methyl	5.94	1.00E-04	0.57	5700	1	0.016	160	2	0.41	4100	1	17	Medium
clofentezine	8.01	1.00E-04	0.32	3200	1	0.1	1000	1	0.015	150	2	18	Medium
cyazofamid	40.9	1.00E-03	0.025	25	4	0.14	140	2	0.14	140	0.1	21	Medium
cycloxydim	98	1.00E-01	44.9	449	2	70.8	708	2	100	1000	1	20	Medium
cymoxanil	99.6	1.00E-01	1.21	12.1	4	27	270	2	29	290	2	31	Medium
cypermethrin	0.0277	1.00E-05	0.1	10000	1	0.0003	30	4	0.69	69000	0	19	Medium
cyprodinil	9.94	1.00E-04	5.2	52000	0	0.033	330	2	2.17	21700	0	8	Low
cyromazine	99.9	1.00E-01	124	1240	1	100	1000	1	90	900	2	18	Medium
diflubenzuron	12.4	1.00E-04	0.3	3000	1	0.0071	71	4	0.2	2000	1	25	Medium
diflufenican	1.37	1.00E-05	10	1000000	0	10	1000000	0	0.0985	9850	1	6	Low
dimethoate	99.5	1.00E-01	90.4	904	2	2	20	4	17.6	176	2	33	Medium
dimethomorph	72.1	1.00E-02	29.2	2920	1	10.6	1060	1	6.2	620	2	18	Medium
dinocap	3.08	1.00E-04	105	1050000	0	0.004	40	4	5.3	53000	0	16	Medium
dithianon	41	1.00E-03	12	12000	0	0.26	260	2	0.1	100	2	19	Medium
diuron	60.9	1.00E-02	0.022	2.2	6	1.4	140	2	6.7	670	2	37	Medium
dodine	96.1	1.00E-01	0.0051	0.051	8	0.13	1.3	8	0.53	5.3	6	89	Very high
esfenvalerate	0.0664	1.00E-05	0.0065	650	2	0.0009	90	4	0.00026	26	4	44	High
famoxadone	2.41	1.00E-04	0.022	220	2	0.012	120	2	0.011	110	2	25	Medium
fenamidone	63.6	1.00E-02	3.84	384	2	0.05	5	6	0.74	74	4	52	High
fenamiphos	35.6	1.00E-03	11	11000	0	0.0019	1.9	8	0.0096	9.6	6	65	High
fenarimol	18.4	1.00E-04	1.5	15000	0	0.82	8200	1	4.1	41000	0	4	Negligible
fenazaquine	0.34	1.00E-05	49	4900000	0	0.0041	410	2	0.0038	380	2	19	Medium
fenebuconazole	39.3	1.00E-03	0.13	130	2	2.3	2300	1	0.68			10	Low
fenoxycarb	8.59	1.00E-04	1.1	11000	0	0.5	5000	1	1.6	16000	0	4	Negligible
fluazifop-P-butyl	1.22	1.00E-05	0.51	51000	0	1	100000	0	1.3	130000	0	0	Negligible
fluazinam	5.64	1.00E-04	0.54	5400	1	0.19	1900	1	0.11	1100	1	13	Low
fludioxonil	9.05E-03	1.00E-05	0.092	9200	1	1.1	110000	0	0.5	50000	0	3	Negligible
flufenacet	4.11E+01	1.00E-03	0.00204	2.04	6	30.9	30900	0	0.2	200	2	29	Medium
flufenoxuron	9.16E+00	1.00E-04	24.6	246000	0	0.00004	0.4	8	0.0049	49	4	54	High
fluquinconazole	3.41E+01	1.00E-03	0.014	14	4	5	5000	1	1.34	1340	1	22	Medium
flusilazole	16.7	1.00E-05	6.4	640000	0	3.4	340000	0	1.2	120000	0	0	Negligible
folpet	46.1	1.00E-03	10	10000	1	1.46	1460	1	0.233	233	2	18	Medium
fosetyl-aluminium	100	1.00E-01	21.9	219	2	100	1000	2	60	600	2	25	Medium
glufosinate-ammonium	99.9	1.00E-01	46.5	465	2	688	6880	1	710	7100	1	16	Medium
glyphosate	100	1.00E-01	1.3	13	4	11	110	2	86	860	2	31	Medium
imazalil	52.3	1.00E-03	0.87	870	2	3.5	3500	1	1.5	1500	1	16	Medium
imidacloprid	99.7	1.00E-01	100	1000	1	85.2	852	2	211	2110	1	17	Medium
indoxacarb	2.41	1.00E-04	0.11	1100	1	0.6	6000	1	0.65	6500	1	13	Low
isoxaben	11.2	1.00E-04	1.4	14000	0	1.3	13000	0	1.1	11000	0	0	Negligible
lambda-cyhalothrin	0.011	1.00E-05	1	100000	0	0.00036	36	4	0.21	21000	0	16	Medium
lufenuron	0.83	1.00E-05	10	1000000	0	0.0011	110	2	29	2900000	0	8	Low
malathion	66.1	1.00E-02	13	1300	0	0.001	0.1	8	0.1	10	4	54	High
mancozebe	99.8	1.00E-01	0.044	0.44	8	3.8	38	4	1	10	4	62	High
mesotrione	100	1.00E-01	3.5	35	4	622	6220	1	120	1200	1	22	Medium
metalaxil-M	95.6	1.00E-01	103	103	2	100	100	2	100	1000	1	20	Medium
methidation	87.4	1.00E-02	22	2200	1	0.0064	0.64	8	0.002	0.2	8	79	High
methiocarb	47.9	1.00E-03	1.15	1.15	8	0.008	0.008	8	0.436	0.436	8	100	Very high
methomyl	99.9	1.00E-01	100	1000	1	0.0076	0.076	8	0.63	6.3	6	68	High
methoxyfenozide	18	1.00E-04	3.4	34000	0	3.7	37000	0	4.2	42000	0	0	Negligible
metribuzin	96.5	1.00E-01	0.021	0.21		49	490	2	74.6	746	2	19	Medium
miclobutanil 736	55.9	1.00E-03	0.91	910	2	17	17000	0	2	2000	1	12	Low
oxamyl	100	1.00E-01	3.3	3.3	6	0.319	0.319	8	4.2	42	4	72	High
oxifluorfen	3.6	1.00E-04	2	20000	0	0.72	7200	1	0.2	2000	1	10	Low
paraquat dichloride	100	1.00E-01	0.00023	0.0023	8	6.1	61	4	26	260	2	51	High
penconazole	17.4	1.00E-04	0.83	8300	1	6.75	67500	0	1.7	17000	0	3	Negligible
phosalone	9.73	1.00E-04	1.1	11000	0	0.00074	7.4	6	0.63	6300	1	30	Medium
phosmet	55.3	1.00E-03	0.07	70	4	0.002	2	6	0.07	70	4	58	High
pirimicarb	95.7	1.00E-01	140	1400	1	0.017	0.17	8	55	550	2	46	High
procymidone	41.9	1.00E-03	2.6	2600	1	1.8	1800	1	7.2	7200	1	13	Low
propamocarb hydrochloride	100	1.00E-01	85	850	2	100	1000	1	92	920	2	21	Medium
propiconazole	17.4	1.00E-04	0.02	200	2	10.2	102000	0	2.6	26000	0	6	Low
propineb	99.9	1.00E-01	2.7	27	4	4.7	47	4	0.4	4	6	61	High
pymetrozine	99.9	1.00E-01	21.6	216	2	87	870	2	100	1000	1	20	Medium
pyrimethanil	61.4	1.00E-02	1.2	120	2	2.9	290	2	10.6	1060	1	20	Medium
quinoxyfen	2.36	1.00E-04	0.058	580	2	0.08	800	2	0.27	2700	1	20	Medium
quizalofop-P -ethyl	2.64	1.00E-04	0.021	210	2	0.29	2900	1	0.5	5000	1	16	Medium
rimsulfuron	100	1.00E-01	0.029	0.29	8	360	3600	1	110	1100	1	34	Medium
spinosad	9.94	1.00E-04	0.09	900	2	14	140000	0	3.5	35000	0	6	Low
spirodiclofen	0.175	1.00E-05	0.06	6000	1	0.051	5100	1	0.035	3500	1	13	Low
tau-fluvalinate	2.85	1.00E-04	10	100000	0	0.0089	89	4	0.0027	27	4	38	Medium
tebuconazole	18	1.00E-04	3.8	38000	0	2.79	27900	0	4.4	44000	0	0	Negligible
tebufenozide	5.84	1.00E-04	0.64	6400	1	3.8	38000	0	3	30000	0	3	Negligible
teflubenzuron	5.19	1.00E-04	0.02	200	2	0.0028	28	4	4	40000	0	22	Medium
terbutilazine	40.5	1.00E-03	0.016	16	4	21.2	21200	0	3.8	3800	1	18	Medium
thiacloprid	42.4	1.00E-03	97	97000	0	85.1	85100	0	25.2	25200	0	0	Negligible
tiabendazole	81.8	1.00E-02	9	900	2	0.81	81	4	0.55	55	4	44	High
tiametoxame	99.9	1.00E-04	100	900	0	100	1000000	0	100	1000000	0	0	Negligible
trifloxystrobin	3.37	1.00E-04	0.0053	53	4	0.011	110		0.015	150	2	23	Medium
trifluralin	1.55	1.00E-05	12.2	1220000	0	0.245	24500	0	0.088	8800	1	6	Low
zirame	98.5	1.00E-01	100	1000	1	0.048	0.48	8	1.9	19	4	57	High
zoxamide	16.1	1.00E-04	0.011	110	2	0.78	7800	1	0.16	1600	1	16	Medium

PEC_{st} - Pesticide concentration in water body ; DT_{50} - half-life period in soil;

LD_{50} - Median lethal dose; EC_{50} - Median effective concentration; PEC-Predicted environmental concentration

Table K.6 *Results from the PRIWS-2 index.*

					TCW	TEW		Algae			*Daphnia*			Fish			Bioaccumulation		Sediment affinity			
Pesticides	MRA	CCW	DT_{50}	Score	(MRA*CCW)/10	TCW*Score DT_{50}	EC_{50}	TER	Score(W)	EC_{50}	TER	Score(W)	LC_{50}	TER	Score(W)	Log_{KOW}	Score (B)	%	Score(S)	PRISW-2	Final score
abamectin	13.5	1.00E-04	28	1	0.000135	0.000135	1000	7407407.41	0	0.00034	2.52	4	0.0032	23.70	2	4.4	1.25	2.06	1.1	25	Medium
acetamiprid	100	1.00E-01	5.4	0.1	1	0.1	93	930.00	1	200	2000.00	0	100	1000.00	1	0.8	1	0.0123	1	5	Negligible
acrinathrin	75	1.00E-05	100	50	0.000075	0.00375	0.82	218.67	1	0.57	152.00	1	5.66	1509.33	0	5.6	1.25	0.234	1	6	Low
alpha-cypermethrin	30	1.00E-05	91	50	0.00003	0.0015	0.1	66.67	2	0.0001	0.07	8	0.0028	1.87	4	6.94	1.25	2.17	1.1	55	High
amitrole	3600	1.00E-02	18	1	3.6	3.6	2.3	0.64	8	6.1	1.69	4	1000	277.78	1	-0.97	1	2.09	1.1	34	High
azoxystrobin	200	1.00E-03	14	1	0.02	0.02	0.12	6.00	4	0.08	4.00	4	0.47	23.50	2	2.5	1.1	1.11	1.1	31	High
benalaxyl	160	1.00E-03	77	10	0.016	0.16	2.4	15.00		0.59	3.69	4	3.75	23.44	2	3.54	1.25	1.65	1.1	25	Medium
benoxacor	75	1.00E-02	5	0.1	0.075	0.0075	0.63	84.00	2	4.8	640.00	1	2.4	320.00	1	2.6	1.1	0.575	1	11	Medium
bentazone	1200	1.00E-01	12	1	12	12	47.3	3.94	4	125	10.42	4	100	8.33	4	-0.46	1	0.000682	1	32	High
bifenthrin	80	1.00E-05	125	50	0.00008	0.004	50	12500.00	0	0.00016	0.04	8	0.00015	0.04	8	6	1.25	2.16	1.1	66	Very high
bupirimate	250	1.00E-04	90	10	0.0025	0.025	1.6	64.00	2	7.3	292.00	1	1.4	56.00	2	3.9	1.25	1.91	1.1	18	Medium
buprofezin	125	1.00E-05	104	50	0.000125	0.00625	2.1	336.00	1	0.42	67.20	2	0.527	84.32	2	4.8	1.25	2.13	1.1	19	Medium
captan	2000	1.00E-02	1	0.01	2	0.02	91	4550.00	0	7.00	350.00	1	0.034	1.70	4	2.8	1.1	0.778	1	17	Medium
carbaryl	1000	1.00E-02	28	1	1	1	1.1	1.10	4	0.006	0.01	8	1.3	1.30	4	1.85	1	0.131	1	44	High
carbendazime	600	1.00E-01	32	10	6	60	419	6.98	4	0.13	0.00	8	0.61	0.01	8	1.51	1	0.0618	1	56	High
chlorpyrifos-methyl	200	1.00E-04	33	10	0.002	0.02	0.57	28.50	2	0.016	0.80	8	0.41	20.50	2	4.24	1.25	2.03	1.1	47	High
cycloxydim	400	1.00E-01	1	0.001	4	0.004	44.9	11225.00	0	70.8	17700.00	0	100	25000	0	1.36	1	0.0442	1	0	Negligible
cyazofamid	80	1.00E-03	5	0.1	0.008	0.0008	0.025	31.25	2	0.14	175.00	1	0.14	0.10	1	3.2	1.1	1.27	1.1	12	Medium
cymoxanil	120	1.00E-01	9	0.1	1.2	0.12	1.21	10.08	2	27	225.00	1	29	241.67	1	0.59	1	0.00763	1	10	Low
cypermethrin	100	1.00E-05	60	10	0.0001	0.001	0.1	100.00	1	0.0003	0.30	8	0.69	690.00	1	6.6	1.25	2.17	1.1	40	High
cyprodinil	300	1.00E-04	45	10	0.003	0.03	5.2	173.33	1	0.033	1.10	4	2.17	72.33	2	4	1.25	1.96	1.1	28	Medium
cyromazine	225	1.00E-01	9.7	0.1	2.25	0.225	124	551.11	1	100	444.44	1	90	400.00	1	-0.061	1	0.00171	1	8	Low
deltamethrin	10	1.00E-05	23	1	0.00001	0.00001	9.1	910000.00	0	0.00056	56.00	2	0.91	91000.00	0	4.6	1.25	0.00529	1	8	Low
dicofol	600	1.00E-04	30	1	0.006	0.006	0.075	12.50	2	0.14	23.33	2	0.183	30.50	2	4.3	1.25	2.06	1.1	22	Medium
diflubenzuron	100	1.00E-04	7	0.1	0.001	0.0001	0.3	3000.00	0	0.0071	71.00	2	0.2	2000.00	0	3.89	1.25	1.9	1.1	8	Low
diflufenican	320	1.00E-05	282	50	0.00032	0.016	10	625.00	1	10	625.00	1	0.0985	6.16	4	4.9	1.25	2.14	1.1	23	Medium
dimethoate	400	1.00E-01	16	1	4	4	90.4	22.60	2	2	0.50	8	17.6	4.40	4	0.704	1	0.00991	1	40	High
dimethomorph	180	1.00E-02	53	10	0.18	1.8	29.2	16.22	2	10.6	5.89	4	6.2	3.44	4	2.63	1.1	0.606	1	31	High
dinocap	192	1.00E-04	24	1	0.00192	0.00192	105	54687.50	0	0.004	2.08	4	5.3	2760.42	0	4.54	1.25	2.1	1.1	17	Medium
dithianon	375	1.00E-03	35	10	0.0375	0.375	12	32.00	2	0.26	0.69	8	0.1	0.27	8	3.2	1.1	1.28	1.1	63	Very high
diuron	1275	1.00E-02	180	50	1.275	63.75	0.022	0.00	8	1.4	0.02	8	6.7	0.11	8	2.85	1.1	0.849	1	70	Very high
dodine	900	1.00E-01	22.3	1	9	9	0.0051	0.00	8	0.13	0.01	8	0.53	0.06	8	1.65	1	0.0845	1	64	Very high
famoxadone	30	1.00E-04	28	1	0.0003	0.0003	0.022	73.33	2	0.012	40.00	2	0.011	36.67	2	4.65	1.25	2.12	1.1	22	Medium
fenamidone	100	1.00E-02	97	50	0.1	5	3.84	0.77	8	0.05	0.01	8	0.74	0.15	8	2.8	1.1	0.79	1	70	Very high
fenamiphos	3400	1.00E-03	1.8	0.001	0.34	0.00034	11	32352.94	0	0.0019	5.59	4	0.0096	28.24	2	3.3	1.1	1.4	1.1	22	Medium
fenazaquine	50000	1.00E-05	45	10	0.05	0.5	49	98.00	2	0.0041	0.01	8	0.0038	0.01	8	5.51	1.25	2.16	1.1	72	Very high
fenbuconazole	75	1.00E-03	306	100	0.0075	0.75	0.13	0.17	8	2.3	3.07	4	0.68	0.91	8	3.23	1.1	1.31	1.1	63	Very high
fenoxycarb	150	1.00E-04	31	10	0.0015	0.015	1.1	73.33	2	0.5	33.33	2	1.6	106.67	1	4.07	1.25	1.99	1.1	18	Medium
fluazinam	200	1.00E-04	26.5	1	0.002	0.002	0.54	270.00	1	0.19	95.00	1	0.11	55.00	2	4.1	1.25	1.4	1.1	15	Medium
fludioxonil	200	1.00E-05	25	1	0.0002	0.0002	0.092	460.00	1	1.1	5500.00	0	0.5	2500.00	0	4.12	1.25	2.35E-03	1	3	Negligible
flufenoxuron	100	1.00E-04	42	10	0.001	0.01	24.6	2460.00	0	0.00004	0.00	8	0.0049	0.49	8	4	1.25	1.80E+00	1.1	66	Very high
fluquinconazole	75	1.00E-03	300	50	0.0075	0.375	0.014	0.04	8	5	13.33	2	1.34	3.57	4	3.24	1.1	1.43E+00	1.1	41	High
flusilazole	40	1.00E-04	95	50	0.0004	0.02	6.4	320.00	1	3.4	170.00	1	1.2	60.00	2	3.74	1.25	1.81	1.1	15	Medium
folpet	1250	1.00E-03	4.3	0.01	0.125	0.00125	10	8000.00	0	1.46	1168.00	0	0.233	186.40	1	3.11	1.1	1.17	1.1	4	Negligible
fosetyl-aluminium	2000	1.00E-01	0.625	0.001	20	0.02	21.9	1095.00	0	100	5000.00	0	60	3000.00	0	-2.4	1	7.83E-06	1	0	Negligible
glufosinate-ammonium	1500	1.00E-01	20	1	15	15	46.5	3.10	4	688	45.87	2	710	47.33	2	0.1	1	2.47E-03	1	20	Medium
Imazalil	375	1.00E-03	5	0.1	0.0375	0.00375	0.87	232.00	1	3.5	933.33	1	1.5	400.00	1	3.82	1.25	1.03	1.1	11	Medium
isoxaben	1000	1.00E-04	120	50	0.01	0.5	1.4	2.80	4	1.3	2.60	4	1.1	2.20	4	3.94	1.25	1.93	1.1	44	High
lambda-cyhalothrin	20	1.00E-05	40	10	0.00002	0.0002	1	5000.00	0	0.00036	1.80	4	0.21	1050.00	0	7	1.25	2.17	1.1	17	Medium
lufenuron	100	1.00E-05	67	10	0.0001	0.001	10	10000.00	0	0.0011	1.10	4	29	29000.00	0	5.12	1.25	2.15	1.1	17	Medium
malathion	3040	1.00E-02	0.18	0.001	3.04	0.00304	13	4276.32	0	0.001	0.33	8	0.1	32.89	2	2.75	1	7.32E-01	1	30	Medium
mancozebe	1650	1.00E-01	1	0.001	16.5	0.0165	0.044	2.67	4	3.8	230.30	1	1	60.61	2	0.26	1	3.57E-03	1	17	Medium
mesotrione	150	1.00E-01	17	1	1.5	1.5	3.5	2.33	4	622	414.67	1	120	80.00	2	-1	1	1.97E-04	1	17	Medium
metalaxil-M	100	1.00E-01	21	1	1	1	103	103.00	1	100	100.00	1	100	100.00	1	1.71	1	9.64E-02	1	8	Low
methidation	630	1.00E-02	18	1	0.63	0.63	22	34.92	2	0.0064	0.01	8	0.002	0.00	8	2.2	1	2.73E-01	1	52	High
methiocarb	150	1.00E-03	35	10	0.015	0.15	1.15	7.67	4	0.008	0.05	8	0.436	2.91	4	3.08	1.1	1.13	1.1	53	High
methomyl	380	1.00E-01	8	0.1	3.8	0.38	100	263.16	1	0.0076	0.02	8	0.63	1.66	4	0.093	1	0.00244	1	38	High
methoxyfenozide	960	1.00E-04	268	50	0.0096	0.48	3.4	7.08	4	3.7	7.71	4	4.2	8.75	4	3.7	1.25	1.78	1.1	44	High
metribuzin	840	1.00E-01	60	10	8.4	84	0.021	0.00	8	49	0.58	8	74.6	0.89	8	1.6	1	7.56E-02	1	64	Very high
oxamyl	2	1.00E-01	7	0.1	0.02	0.002	3.3	1650.00	0	0.319	159.50	1	4.2	2100.00		-0.44	1	0.000714	1	3	Negligible
oxifluorfen	960	1.00E-04	55	10	0.0096	0.096	2	20.83	2	0.72	7.50	4	0.2	2.08	4	4.47	1.25	2.09	1.1	39	High
phosalone	600	1.00E-04	4	0.001	0.006	0.000006	1.1	183333.33	0	0.00074	123.33	1	0.63	105000.00	0	4.01	1.25	1.96	1.1	4	Negligible
phosmet	300	1.00E-03	7	0.1	0.03	0.003	0.07	23.33	2	0.002	0.67	8	0.07	23.33	2	2.95	1.1	9.70E-01	1	37	High
pirimicarb	375	1.00E-01	234	50	3.75	187.5	140	0.75	8	0.017	0.00	8	55	0.29	8	1.7	1	9.43E-02	1	64	Very high
procymidone	700	1.00E-03	84	10	0.07	0.7	2.6	3.71	4	1.8	2.57	4	7.2	10.29	2	3.14	1.1	1.14	1.1	31	High
propamocarb hydrochlorid	722	1.00E-01	30	1	7.22	7.22	85	11.77	2	100	13.85	2	92	12.74	2	-1.21	1	1.21E-04	1	16	Medium
propiconazole	50	1.00E-04	70	10	0.0005	0.005	0.02	4.00	4	10.2	2040.00	0	2.6	520.00	1	3.72	1.25	1.79	1.1	15	Medium
propineb	1750	1.00E-01	3	0.001	17.5	0.0175	2.7	154.29	1	4.7	268.57	1	0.4	22.86	2	-0.26	1	1.08E-03	1	11	Medium
pymetrozine	300	1.00E-01	69	10	3	30	21.6	0.72	8	87	2.90	4	100	3.33	4	-0.18	1	1.30E-03	1	40	High
pyrimethanil	300	1.00E-02	54	10	0.3	3	1.2	0.40	8	2.9	0.97	8	10.6	3.53	4	2.84	1.1	8.37E-01	1	57	High
quinoxyfen	50	1.00E-04	454	100	0.0005	0.05	0.058	1.16	4	0.08	1.60	4	0.27	5.40	4	4.66	1.25	2.12	1.1	44	High
quizalofop-P -ethyl	150	1.00E-04	0.91667	0.001	0.0015	0.0000015	0.021	14000.00	0	0.29	193333.33	0	0.5	333333.33	0	4.61	1.25	2.11	1.1	0	Negligible
rimsulfuron	15	1.00E-01	20	1	0.15	0.15	0.029	0.19	8	360	2400.00	0	110	733.33	1	-1.47	1	6.67E-05	1	19	Medium
spirodiclofen	960	1.00E-05	7.3	0.1	0.00096	0.000096	6	62500.00	0	0.051	531.25	1	0.035	364.58	1	5.8	1.25	2.17	1.1	8	Low
tau-fluvalinate	144	1.00E-04	92	50	0.00144	0.072	10	138.89	1	0.0089	0.12	8	0.0027	0.04	8	4.26		1.02		0	Negligible
tebuconazole	100	1.00E-04	28	1	0.001	0.001	3.8	3800.00	0	2.79	2790.00	0	4.4	4400.00		3.7	1.25	1.78	1.1	0	Negligible
tebufenozide	144	1.00E-04	348	100	0.00144	0.144	0.64	4.44		3.8	26.39	2	3	20.83	2	4.25	1.25	2.05	1.1	17	Medium
terbutilazine	2415	1.00E-03	60	10	0.2415	2.415	0.016	0.01	8	21.2	8.78	4	3.8	1.57	4	3.56	1.25	1.29	1.1	55	High
thiacloprid	96	1.00E-03	7	0.1	0.0096	0.00096	97	101041.67	0	85.1	88645.83	0	25.2	26250.00	0	1.26	1	1.25E+00	1.1	0	Negligible
thiamethoxam	75	1.00E-04	51	10	0.00075	0.0075	100	13333.33	0	100	13333.33	0	100	13333.33	0	-0.13	1	0.00146	1	0	Negligible
zoxamide	149.4	1.00E-04	10	1	0.001494	0.001494	0.011	7.36	4	0.78	522.09	1	0.16	107.10	1	3.76	1.25	1.82	1.1	19	Medium

TER- Toxicity Exposure Ratio; CCW- Classes of water concentration; TEW -Theorical exposure in water; TCW-Theorical concentration in water; Log_{KOW}-Logarithm of the partition coefficient octanol/water; DT_{50}-half-life; LD_{50}-Median lethal dose; EC_{50}-Median effective concentration; MRA-Maximum application rate

Table K.7 *Results from the ERIP index.*

	FUGACITY								Tx WATER														Tx EPYGEAN				Tx HYPOGEAN							
	Air affinity		Water affinity		Soil affinity		Sediment affinity		Persistence		Bioaccumulation		MRA		Algae		*DAPHNIA*		Fish		Plants		Bees		Beneficial arthropods		Birds		Mammals			Earthworms				
Pesticides	%	Score(DA)	%	Score(DW)	%	Score(DS)	%	Score (Dsed)	DT_{50}	Score(P)	LOG_{KOW}	Score(B)	g/ha	Score	EC_{50}	Score	EC_{50}	Score	LC_{50}	Score	Score(A)	LD_{50}	Score(B)	ScoreC	LC_{50}	Score(D)	NOEL	Score(E)	LC_{50}	Score(A)	Score(B)	ICRA	Final score
abamectin	0.395	1	4.17	1	92.9	2	2.06	1	28	1	4.4	2	13.5	0.5	1000	0.5	0.00034	2	0.0032	2	0.1	0.0022	2	2	84.6	1.5	4.5	1.5	28	1	0.1	6	Negligible
acetamiprid	1.07E-06	0.5	99.4	2	0.556	1	0.0123	0.5	5.4	0.5	0.8	1	100	1	93	1	200	0.1	100	0.5	0.1	14.5	0.5	0.5	98	1.5	15	1	9	1.5	0.1	3	Negligible
alpha-cypermethrin	0.00627	0.5	0.0127	0.5	97.7	2	2.17	1	91	3	6.94	2	30	0.5	0.1	2	0.0001	2	0.0028	2	0.1	0.059	2	2	2025	1	1.5	1.5	100	1	0.1	17	Low
azoxystrobin	6.91E-08	0.5	46.1	1.25	49.8	2	1.11	1	14	1	2.5	1.1	200	1	0.12	2	0.08	2	0.47	1.5	0.1	25	0.5	0.5	2000	1	18	1	283	1	0.1	7	Negligible
benalaxyl	0.0372	0.5	24.1	1.25	74.1	2	1.65	1	77	2	3.54	2	160	1	2.4	1.5	0.59	1.5	3.75	1	0.1	100	0.5	0.5	4500	1	100	1	180	1	8	22	Medium
benalaxyl-M	0.0023	0.5	19.1	1.25	79.1	2	1.76	1	124	3	3.67	2	100	1	16.5	1	17	0.1	4.9	1	0.1	104	0.5	0.5	2000	1	4.4	1.5	472.7	1	8	33	Medium
bentazone	4.67E-05	0.5	100	2	0.0307	0.5	0.000682	0.5	12	1	-0.46	1	1200	3	47.3	1	125	0.1	100	0.5	2	100	0.5	0.5	1140	1	10	1.5	870	1	0.1	4	Negligible
bifenthrin	0.228	1	0.11	0.5	97.1	2	2.16	1	125	3	6	2	80	1	50	1	0.00016	2	0.0002	2	0.1	0.1	1.5	1.5	1800	1	1.5	1.5	1000	1	0.1	33	Medium
bupirimate	0.00606	0.5	12.2	1.25	85.8	2	1.91	1	90	2	3.9	2	250	2	1.6	1.5	7.3	1	1.4	1	0.1	50	0.5	0.5	2700	1	15	1	1000	1	8	44	High
buprofezin	0.0117	0.5	1.72	1	96.1	2	2.13	1	104	3	4.8	2	125	1	0.1	1.5	0.42	1.5	0.527	1.5	0.1	163.5	0.1	0.1	2000	1	0.9	2	500	1	0.1	33	Medium
carbaryl	0.00133	0.5	94	2	5.89	1.25	0.131	1	28	1	1.85	1	1000	3	1.1	1.5	0.006	2	1.3	1	0.1	1	1.5	1.5	1000	1.5	200	1	106	1	0.1	6	Negligible
carbendazime	0.0714	0.5	97.1	2	2.78	1	0.0618	0.5	32	2	1.51	1	600	2	419		0.13	1.5	0.61	1.5	0.1	50	0.5	0.5	5826	1	300	1	6	1.5	8	6	Negligible
carbofuran	0.000992	0.5	97.1	2	2.85	1	0.0633	0.5	60	2	1.52	1	500	2	6.5	1.5	0.0386	2	1.75	1	0.1	0.04	2	8	2.5	2	10	1.5	13	1	0.1	6	Negligible
chlorothalonil	2.91E-01	1	56.9	1.5	41.9	2	0.931	1	28	1	2.92	1.1	1500	3	0.21	2	0.07	2	0.047	2	0.1	63	0.5	0.5	4640	1	3	1.5	268.5	1	8	8	Negligible
chlorpyrifos	2.98E-01	1	2.15	1	95.4	2	2.12	1	56	2	4.7	2	960	2	0.4	2	0.0017	2	0.0007	2	0.1	0.059	2	2	32	1.5	0.1	2	210	1	0.1	44	High
chlorpyrifos-methyl	0.454	1	5.94	1	91.5	2	2.03	1	33	2	4.24	2	200	1	0.57	2	0.016	2	0.41	1.5	0.1	0.38	1.5	1.5	932	1.5	0.1	2	182	1	0.1	22	Medium
cycloxydim	0.00123	0.5	98	2	1.99	1	0.0442	0.5	1	0.5	1.36	1	400	2	44.9	1	70.8	0.5	100	0.5	2	100	0.5	0.5	2000	1	7	1.5	1000	1	0.1	3	Negligible
cymoxanil	0.000779	0.5	99.6	2	0.343	1	0.00763	0.5	9	0.5	0.59	1	120	1	1.21	1.5	27	0.5	29	0.5	0.1	25	0.5	0.5	2250	1	3	1.5	2208	0.5	8	3	Negligible
cyazofamid	0.331	1	40.9	1.25	57.4	2	1.27	1	5	0.5	3.2	1.1	80	1	0.025	2	0.14	1.5	0.14	1.5	0.1	151.7	0.1	0.1	5000	1	17	1	1000	1	8	4	Negligible
cypermethrin	0.000118	0.5	0.0277	0.5	97.7	2	2.17	1	60	2	6.6	2	100	1	0.1	2	0.0003	2	0.69	1.5	0.1	0.035	2	2	2000	1	0.05	2	100	1	0.1	22	Medium
cyromazine	1.17E-06	0.5	99.9	2	0.0769	0.5	0.00171	0.5	9.7	0.5	-0.061	1	225	2	124		100	0.5	90	0.5	0.1	186	0.1	0.1	1000	1.5	300	1	1000	1	0.1	3	Negligible
diclofol	0.0264	0.5	5.24	1	92.6	2	2.06	1	30	1	4.3	2	600	2	0.075	2	0.14	1.5	0.183	1.5	0.1	50	0.5	0.5	1418	1	0.22	2	43.1	1	0.1	22	Medium
diflubenzuron	0.00119	0.5	12.4	1.25	85.6	2	1.9	1	7	0.5	3.89	2	100	1	0.3	2	0.0071	2	0.2	1.5	0.1	100	0.5	0.5	2000	1	40	1	500	1	0.1	6	Negligible
diflufenican	0.00942	0.5	1.37	1	96.4	2	2.14	1	282	3	4.9	2	320	2	10	1.5	10	1	0.0985	2	2	100	0.5	0.5	2150	1	1000	1	500	1	0.1	65	Very high
dimethoate	0.000502	0.5	99.5	2	0.446	1	0.00991	0.5	16	1	0.704	1	400	2	90.4	1	2	1	17.6	0.5	0.1	0.15	1.5	1.5	14.1	1.5	0.2	2	99.5	1	0.1	4	Negligible
dimethomorph	0.000113	0.5	72.1	1.5	27.2	1.5	0.606	1	53	2	2.63	1.1	180	1	29.2	1	10.6	0.5	6.2	1	0.1	32.4	0.5	0.5	2000	1	9	1.5	31	1	8	5	Negligible
dinocap	0.00508	0.5	3.08	1	94.6	2	2.1	1	24	1	4.54	2	192	1	105	0.5	0.004	2	5.3	1	0.1	6.5	1	1	2150	1	0.4	2	120	1	8	12	Low
dithianon	0.000048	0.5	41	1.25	57.6	2	1.28	1	35	2	3.2	1.1	375	2	12	1	0.26	1.5	0.1	1.5	0.1	25.4	0.5	0.5	290	1.5	2.8	1.5	578	1	8	24	Medium
dodine	8.99E-05	0.5	96.1	2	3.8	1	0.0845	0.5	22.3	1	1.65	1	900	2	0.0051	2	0.13	1.5	0.53	1.5	0.1	0.2	1.5	1.5	788	1.5	800	1	547	1	8	4	Negligible
esfenvalerate	0.000572	0.5	0.0664	0.5	97.6	2	2.17	1	287	3	6.22	2	15	0.5	0.0065	2	0.0009	2	0.0003	2	0.1	0.06	2	2	381	1.5	2	1.5	212.5	1	0.1	17	Low
famoxadone	0.00228	0.5	2.41	1	95.4	2	2.12	1	28	1	4.65	2	30	0.5	0.022	2	0.012	2	0.011	2	0.1	25	0.5	0.5	2250	1	1.2	1.5	470	1	8	6	Negligible
fenamidone	0.000177	0.5	63.6	1.5	35.6	2	0.79	1	97	3	2.8	1.1	100	1	3.84	1.5	0.05	2	0.74	1.5	0.1	159.8	0.1	0.1	2000	1	3.6	1.5	25	1	8	8	Negligible
fenazaquine	3.30E-04	0.5	0.34	0.5	97.4	2	2.16	1	45	2	5.51	2	50000	4	49	1	0.0041	2	0.0038	2	0.1	1.21	0.1	0.1	1747	1	0.5	2	26.5	1	0.1	86	Very high
fenbuconazole	2.45E-01	1	39.3	1.25	59.1	2	1.31	1	306	3	3.23	1.1	75	1	0.13	2	2.3	1	0.68	1.5	0.1	5.2	1	1	2110	1	6.4	1.5	100	1	8	19	Low
fenoxycarb	5.83E-05	0.5	8.59	1	89.4	2	1.99	1	31	2	4.07	2	150	1	1.1	1.5	0.5	1.5	1.6	1	0.1	0.1	1.5	1.5	7000	1	5.5	1.5	425	1	0.1	22	Medium
fluazinam	2.99E+01	2	5.64	1	62.9	2	1.4	1	26.5	1	4.1	1.25	200	1	0.164	2	0.22	1.5	0.036	2	0.1	100	0.5	1	1782	1	3.48	1.5	1000	1	8	8	Negligible
fludioxonil	99.9	1	9.05E-03	0.5	0.1	1.0	0.0	0.5	25	1	4.12	2	200	1	0.092	2	1.1	1	0.5	1.5	0.1	329	0.1	0.1	2000	1	40	1	1000	1	8	6	Negligible
flufenacet	4.3E-03	0.5	41.1	1.25	57.6	2.0	1.3	1	32	2	3.2	1.1	600	2	0.00204	2	30.9	0.5	1608	0.1	2	170	0.1	0.1	1608	1	1.67	1.5	219	1	0.1	24	Medium
flufenoxuron	1.40E-05	0.5	9.2	1	81.1	2.0	1.8	1	42	2	4	2	100	1	24.6	1	0.00004	2	0.0049	2	0.1	109.1	0.1	0.1	2000	1			1000	1	0.1	22	Medium
fluquinconazole	1.46E-05	0.5	34.1	1.25	64.4	2.0	1.4	1	300	3	3.24	1.1	75	1	0.014	2	5	1	1.34	1	0.1	100	0.5	0.5	2000	1	0.31	2	1000	1	8	19	Low
flusilazole	7.82E-04	0.5	16.7	1.25	81.4	2.0	1.8	1	95	3	3.74	2	40	0.5	6.4	1.5	3.4	1	1.2	1	0.1	150	0.1	0.1	1590	1	10	1.5	388	1	8	17	Low
folpet	7.36E-02	0.5	46.1	1.25	52.6	2.0	1.2	1	4.3	0.5	3.11	1.1	1250	3	10	1.5	1.46	1	0.233	1	0.1	236	0.1	0.1	2000	1	44.5	1.5	1000	1	8	10	Negligible
fosetyl-aluminium	6.53E-09	0.5	100	2	0.0004	0.5	0.0	0.5	0.625	0.5	-2.4	1	2000	3	21.9	1	100	0.5	60	0.5	0.1	461.8	0.1	0.1	8000	1	298	1	1000	1	8	3	Negligible
glyphosate	2.39E-07	0.5	100	2	0.0	0.5	0.0	0.5	130	3	-3.7	1	900	2	1.3	1.5	11	0.5	86	0.5	2	100	0.5	0.5	3851	1	410	1	480	1	0.1	5	Negligible
isoxaben	2.97E-04	0.5	11.2	1.25	86.8	2	1.93	1	120	3	3.94	1.25	1000	3	1.4	1.5	1.3	1	1.1	1	0.1	100	0.5	0.5	2000	1	5.6	1.5	500	1	0.1	61	Very high
lambda-cyhalothrin	4.07E-05	0.5	0.011	0.5	97.7	2	2.17	1	40	2	7	2	20	0.5	1	1.5	0.00036	2	0.21	1.5	0.1	0.038	2	2	3950	1	0.5	2	1000	1	0.1	12	Low
lufenuron	5.80E-03	0.5	0.83	0.5	96.9	2	2.15	1	20	1	5.12	2	100	1	10	1.5	0.0011	2	29	0.5	0.1	197	0.1	0.1	2000	1	2	1.5	1000	1	0.1	12	Low
malathion	1.60E-01	0.5	66.1	1.5	32.900	2	7.32E-01	1	0.18	0.5	2.75	1.1	3040	3	13	1	0.001	2	0.1	1.5	0.1	0.13	1.5	1.5	211	1	500	1	613	1	0.1	5	Negligible
mancozebe	1.19E-02	0.5	99.8	2	0.161	1	3.57E-03	0.5	1	0.5	0.26	1	1650	3	0.044	2	3.8	1	1	1.5	0.1	209	0.1	0.1	1290	1	4.8	1.5	1000	1	8	4	Negligible
mesotrione	1.80E-05	0.5	100	2	0.0088	0.5	1.97E-04	0.5	17	1	-1	1	150	1	3.5	1.5	622	0.5	120	0.5	2	11	1	1	2000	1	0.24	2	437.7	1	0.1	3	Negligible
metalaxil-M	6.95E-04	0.5	95.6	2	4.34	1	9.64E-02	0.5	21	1	1.71	1	100	1	103	0.5	100	0.5	100	0.5	0.1	127	0.1	0.1	981	1.5	250	1	830	1	8	3	Negligible
methidation	6.78E-03	0.5	87.4	1.5	12.3	1.5	2.73E-01	1	18	1	2.2	1	630	2	22	1	0.0064	2	0.002	2	0.1	0.13	1.5	1.5	23.6	1.5	4	1.5	5.6	1.5	0.1	5	Negligible
methiocarb	1.23E-03	0.5	47.9	1.25	51	2	1.13	1	35	2	3.08	1.1	150	1	1.15	1.5	0.008	2	0.436	1.5	0.1	0.23	1.5	1.5	7.1	2	60	1	200	1	0.1	13	Low
methomyl	4.13E-05	0.5	99.9	2	0.11	1	0.00244	0.5	8	0.5	0.093	1	380	2	100	1	0.0076	2	0.63	1.5	0.1	0.28	1.5	1.5	24.2	1.5	50	1	21	1	0.1	3	Negligible
methoxyfenozide	6.12E-04	0.5	18	1.25	80.1	2	1.78	1	268	3	3.7	2	960	2	3.4	1.5	3.7	1	4.2	1	0.1	100	0.1	0.1	2250	1	10	1.5	1213	0.5	0.1	65	Very high
metribuzin	2.34E-04	0.5	96.5	2	3.4	1	7.56E-02	0.5	60	2	1.6	1	840	2	0.021	2	49	0.5	74.6	0.5	2	35	0.5	0.5	164	1.5	100	1	331.8	1	0.1	6	Negligible
oxamyl	8.19E-06	0.5	100	2	0.0321	0.5	0.000714	0.5	7	0.5	-0.44	1	2	0.5	3.3	1.5	0.319	1.5	4.2	1	0.1	0.38	1.5	1.5	3.16	2	50	1	112	1	8	2	Negligible
oxifluorfen	6.15E-02	0.5	3.6	1	94.1	2	2.09	1	55	2	4.47	2	960	2	2	1.5	0.72	1.5	0.2	1.5	2	100	0.5	0.5	2150	1	2	1.5	1000	1	0.1	44	High
paraquat dichloride	8.51E-08	0.5	100	2	8.86E-05	0.5	1.97E-06	0.5	2800	4	-4.5	1	1100	3	0.00023	2	6.1	1	26	0.5	2	36	0.5	2	175	1	0.6	2	1380	0.1	0.1	8	Negligible
penconazole	2.36E-03	0.5	17.4	1.25	80.8	2	1.79	1	343	4	3.72	2	35	0.5	0.83	2	6.75	1	1.7	1	0.1	5	1	1	1590	1	0.71	2	1000	1	8	22	Medium
phosalone	1.44E-02	0.5	9.73	1	88.2	2.0	2.0	1	4	0.5	4.01	2	600	2	1.1	1.5	0.00074	2	0.63	1.5	0.1	4.5	1	1	2150	1	2.5	1.5	22.5	1	0.1	12	Low
phosmet	9.36E-03	0.5	55.3	1.5	43.7	2.0	1.0	1	7	0.5	2.95	1.1	300	2	0.07	2	0.002	2	0.07	2	0.1	1	1.5	1.5	57	1.5	40	1	52	1	0.1	4	Negligible
pirimicarb	6.24E-04	0.5	95.7	2	4.25	1	9.43E-02	0.5	234	3	1.7	1	375	2	140	0.5	0.017	2	55	0.5	0.1	4	1	1	20.9	1.5	3.5	1.5	60	1	0.1	8	Negligible
procymidone	5.7	1.5	41.9	1.25	51.2	2	1.14	1	84	2	3.14	1.1	700	2	2.6	1.5	1.8	1	7.2	1	0.1	100	0.5	0.5	4092	1	300	1	1000	1	8	25	Medium
propamocarb hydrocl	3.50E-07	0.5	100	2	5.46E-03	0.5	1.21E-04	0.5	30	1	-1.21	1	722	2	85	1	100	0.5	92	0.5	0.1	84	0.5	0.5	1842	1	26	1	660	1	8	3	Negligible
propiconazole	3.29E-04	0.5	17.4	1.25	80.8	2	1.79	1	70	2	3.72	2	50	1	0.02	2	10.2	0.5	2.6	1	0.1	100	0.1	0.1	2223	1	3.6	1.5	686	1	8	22	Medium
propineb	7.09E-07	0.5	99.9	2	0.0487	0.5	0.00108	0.5	3	0.5	-0.26	1	1750	3	2.7	1.5	4.7	1	0.4	1.5	0.1	70	0.5	0.5	5000	1	50	1	700	1	0.1	3	Negligible
pymetrozine	6.14E-05	0.5	99.9	2	0.0585	0.5	1.30E-03	0.5	69	2	-0.18	1	300	2	21.6	1	87	0.5	100	0.5	0.1	117	0.1	0.1	2000	1	3.7	1.5	250	1	0.1	4	Negligible
pyrimethanil	4.57E-02	0.5	61.4	1.5	37.6	2	8.37E-01	1	54	2	2.84	1.1	300	2	1.2	1.5	2.9	1	10.6	0.5	0.1	100	0.5	0.5	2000	1	20	1	625	1	8	10	Low
quinoxyfen	0.038	0.5	2.36	1	95.4	2	2.12	1	454	4	4.66	1.25	50	1	0.058	2	0.08	2	0.27	1.5	0.1	100	0.5	0.5	2250	1	20	1	923	1	8	28	Medium
rimsulfuron	0.00133	0.5	100	2	0.003	0.5	6.67E-05	0.5	20	1	-1.47	1	15	0.5	0.029	2	360	0.1	110	0.1	2	100	0.5	0.5	2000	1	3000	0.5	1000	1	0.1	2	Negligible
spinosad	1.97E-07	0.5	9.94	1	88	2	1.94	1	0.5	0.5	4	2	120	1	0.09	2	14	0.5	3.5	1	0.1	0.0029	1.5	1.5	2000	1	9	1.5	1000	1	0.1	4	Negligible
tebuconazole	5.38E-05	0.5	18	1.25	80.1	2	1.78	1	28	1	3.7	2	100	1	3.8	1.5	2.79	1	4.4	1	0.1	83	0.5	0.5	1988	1	20	1	1381	0.5	8	12	Low
tebufenozide	7.94E-05	0.5	5.84	1	92	2	2.05	1	348	4	4.25	2	144	1	0.23	2	3.8	1	3	1	0.1	234	0.1	0.1	2150	1	5.5	1.5	1000	1	0.1	44	High
terbutilazine	3.37E-02	0.5	40.5	1.25	58.1	2	1.29	1	60	2	3.21	2	2415	3	0.016	2	21.2	0.5	3.8	1	0.1	100	0.5	0.5	1000	1.5	15.4	1	200	1	0.1	65	Very high
thiacloprid	3.56E-09	0.5	42.4	1.25	56.3	2	1.25E+00	1	7	0.5	1.26	1	96	1	97	1	85.1	0.5	25.2	0.5	0.1	17.32	0.5	0.5	49	1.5	1.23	1.5	105	1	0.1	4	Negligible
trifluralin	3.39	1.5	1.55	1	92.9	2	2.06	1	126	3	4.83	2	1200	3	12.2	1	0.245	1.5	0.088	2	2	100	0.5	0.5	2000	1	813	1	1000	1	0.1	97	Very high
zoxamide	0.0163	0.5	16.1	1.25	82	2	1.82	1	10	1	3.76	2	149.4	1	0.011	2	0.78	1.5	0.16	1.5	0.1	100	0.5	0.5	2000	1	50	1	1070	0.5	8	12	Low

LD50-Median lethal dose; NOEL-No-observed-effect-level; EC50-Median effective concentration;

NOEL-No-observed-effect-level; MRA-Maximum application rate; DT50-half-life; LogKOW-logarithm of the partition coefficient octanol/water